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PRELIMINARY STUDY OF RÔLE OF SALTS IN POLLEN TUBE GROWTH[1]

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The primary purpose of this paper is to present the results of some preliminary investigations concerning the relation of electrolytes to pollen tube growth. Those who have attempted to cultivate pollen *in vitro*, and have sought to improve their culture media by the addition of salts commonly employed in nutrient solutions, will have been impressed with the highly toxic nature of these substances. No case has been reported, so far as the writer is aware, in which it was definitely shown that the addition of salts, either singly or in mixtures, promotes pollen tube growth. While high germination and strong initial growth have been obtained with certain forms in culture solutions containing sucrose only, for example, it is not to be inferred that salts are unimportant in this connection. Indeed, the present evidence points to their being of considerable significance. Before proceeding to a discussion of the specific effects of electrolytes, however, the status of certain other problems in pollen physiology, to which the present investigations are more or less intimately related, will be indicated briefly.

The male gametophyte of angiosperms is dependent during growth upon the pistillate tissue it traverses, in so far as the materials stored in the microspore are insufficient for its complete

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development. It would be interesting to know to what extent the materials contributed by the spore must be supplemented. Are the contents of the mature pollen grain so completely organized that the developing gametophyte requires only carbohydrates, these to serve as building materials for the tube structure and as a source of energy? Or are nitrogenous substances demanded as well? What rôle do salts play in maintaining a favorable relation for growth? Studies on artificial media should provide answers to these questions.

Numerous investigations have shown that the pollen of many plants may be germinated in artificial media of relatively simple composition. Indeed, the pollen of some forms requires only suitable moisture conditions for the production of tubes, as VON MOHL (15) first observed, and as JOST (9), MARTIN (13), and ANTHONY and HARLAN (2) have amply confirmed. The pollen of a great many other species may be germinated readily in sugar solutions, especially sucrose solutions. That the rôle of the sugars is not purely a physical one is shown by the fact that certain kinds of pollen that germinate in a cane sugar medium of a particular concentration will not do so in isotonic solutions of glucose or fructose. The writer has found, for instance, that sweet pea pollen gives a high percentage of germination in 0.418M sucrose, but none whatever in glucose or fructose, or mixtures of these in the same molar concentration. This indicates that the production of tubes may be dependent upon a definite chemical stimulus. It is probable that sucrose plays a wide rôle in this connection. MOLISCH (14) observed that the pollen of *Azalea indica*, *Rhododendron ponticum*, and *R. arboreum*, which otherwise would germinate only on the stigma, grew well in weak solutions of malic acid or calcium malate.

The two most important conditions for germination seem to be the maintenance of certain water relations between the pollen grain and its substratum, and the presence, in many cases at least, of a definite chemical stimulus. The chemical stimuli are probably limited to a few widely distributed substances, among which cane sugar appears to be of common occurrence. It has been found by various investigators that pollen of many species germinates on foreign stigmas. This shows that the requirements for germination of pollen from widely different species may not vary greatly.

It is a much simpler matter to induce pollen to germinate on artificial media than it is to maintain the growth of the tubes. In consequence our knowledge of the requirements for complete development is rather meager. STRASBURGER'S (**18**) observation that the pollen tubes of certain plants would grow readily in the pistils of some very different forms suggests that here too specificity may not be great. The data are far too few at present, however, to establish any conclusion on this point.

The rapidity with which the starchy or fatty reserve materials contributed by the pollen grain are digested in the growing tube on artificial media shows that the carbon requirement of the male gametophyte is comparatively high. This becomes intelligible when the relative extent of the wall of the tube secreted is considered. The carbon supply may be the most important element in the nutrition of the microgametophyte.

Little is known regarding the nitrogen requirements of pollen tubes. Possibly, where the gametes are formed in the pollen grain before the latter is shed by the parent plant, and this occurs in certain species among both dicotyledons and monocotyledons (**5**), the gametophyte may be independent of additional supplies of nitrogen. The mass of protoplasm at no time becomes large, but advances with the tip of the elongating tube, the older portions of the latter being frequently cut off by definite cross-walls, the *Propfen* of STRASBURGER. It is generally believed that of the principal cell parts, the nucleus is the richest in proteins. If the male nuclei are formed at an early period in the history of the gametophyte, the demand for further nitrogen from the conducting tissue may not be important. This may not be true, of course, when the generative nucleus divides in the tube.

Such analyses as are available indicate that the nitrogen content of pollen is relatively high. CZAPEK (**6**) may be consulted for a summary of the earlier investigations. HEYL (**8**) found 18 per cent non-protein nitrogenous substance and 30 per cent albuminous material in rye pollen. The same investigator has shown that ragweed pollen, with its three nuclei, contains 24.4 per cent protein. ANDERSON and KULP (**1**) report that air dried maize pollen contains 4.3 per cent nitrogen. If we employ the factor 6.25 (a rather

arbitrary value in the present case), this is equivalent to 26.87 per cent protein. A definite conclusion regarding the adequacy of the nitrogen stored within the pollen grain for complete development of the gametophyte must await the results of cultural studies on nitrogen-free artificial media.

A systematic investigation of the rôle of salts in pollen tube growth, in keeping with the advances in our knowledge of the general physiological importance of these substances, has never been made. Some results have been published, however, which indicate that their addition to artificial media may be very injurious. LIDFORSS (**10**) found that NaCl, KNO_3, and $Ca(NO_3)_2$, even in very small amounts, are poisonous to pollen, although different species vary in the amount of injury shown. In a later paper he (**11**) reports that egg albumin is toxic, but after dialysis will exert a chemotropic effect upon certain kinds of pollen tubes. He attributes the injurious effects of the undialyzed substance to the salts present. TOKUGAWA (**19**) tested the influence of KNO_3, K_3PO_4, $CaSO_4$, $FeSO_4$, $ZnSO_4$, and $CuSO_4$, and found all of them toxic to pollen in varying degrees. PATON (**17**) reports that good growth of pollen from several species was obtained on a medium containing 0.05 per cent $MgSO_4$, 0.1 per cent K_2HPO_4, 0.05 per cent KCl, 0.01 per cent $FeSO_4$, and 0.2 per cent agar. No data are presented, however, which show the amount of growth obtained.

While previous investigators have shown that pollen is very sensitive to various neutral salts, but little information has been gained regarding their relative toxicities or the minimum concentrations in which injury is effected. No information has heretofore been available by which the importance of such a variable in artificial media could be determined, nor has any method been suggested by which the toxicity might be overcome. Moreover, nothing has been learned with respect to the rôle of salts in the preservation of the normal permeability of the pollen tube, a matter of paramount importance, perhaps, in cultural methods.

The present paper constitutes little more than a preliminary survey of the subject. Those who have worked in this field are aware of the difficulties involved in getting evidence for an interpretation of the phenomena in quantitative terms. The extraor-

dinary variability in the responses of pollen under seemingly constant conditions in artificial media remains largely unexplained. Attention has been called to this situation in previous papers (**3, 4**). Under the circumstances large numbers must be used and frequent repetitions made in order to secure significant data. Laborious as this may be, no other way has been found as yet by which the shortcomings of the material in this regard may be circumvented. It seems probable that in several of the experiments here reported significant differences in germination or growth occur as the result of known differential factors in the media; the magnitudes of these differences, however, are subject to large fluctuations due to the vagaries of the material, and the values obtained are to be interpreted with this in mind.

Materials and methods

In the majority of the experiments reported here sweet pea pollen was employed. It is readily procurable in good condition, and usually gives a high percentage of germination under appropriate circumstances. Fifteen per cent c.p. sucrose, in water which had been carefully redistilled into Pyrex glass, formed the basis of the culture media. In view of the probable toxic effect of impurities, the highest grades of chemicals were employed. The pollen was grown in sealed drop cultures at a temperature of 25° C., except in a few of the earlier tests when a temperature control chamber was not available. In each set a check culture containing only sucrose was included. Measurements of pollen tube length were made with an ocular micrometer at a magnification of about 75 diameters. In most of the experiments in which sweet pea pollen was used, the influence of the various salts added was determined by their effect on the amount of growth. Germination counts were made in only a few cases, but in further experiments these values should be regularly obtained also. The *Nicotiana sanderae* pollen employed in the later experiments was so badly shrunken in many cases, and so variable in behavior, that it did not seem pertinent to attempt to secure any more than qualitative data with it. It was hoped, however, that the evidence obtained, taken in conjunction with that from the sweet pea, might serve for the recognition of the more

important issues of the problem, and indicate along what lines quantitative experiments might prove most profitable.

Toxicity of sodium, potassium, and lithium salts

The amounts of growth of sweet pea pollen tubes in cultures containing 15 per cent sucrose and various quantities of NaCl are shown in table I. It will be seen that 0.01M NaCl is so toxic that growth was entirely inhibited at that concentration in two tests, and very greatly reduced in the remainder. At 0.001M the average length of the tubes was less than half that in the salt free check culture. Some toxicity is still in evidence when the amount of NaCl in the media is further reduced fivefold, that is, at 0.0002 M, although the results in Test 2 of the experiment are not in conformity with this.

TABLE I

LENGTH IN MICRONS OF POLLEN TUBES GROWN IN SUCROSE CULTURES CONTAINING NaCl AS STATED

TEST	CHECK		0.01M NaCl		0.001M NaCl		0.0002M NaCl	
	No.	Length	No.	Length	No.	Length	No.	Length
1	160	234	85	39	119	126	137	195
2	96	152	35	36	70	87	73	250
3	96	284	0	0	60	69	95	155
4	120	208	0	0	96	85	96	110
Average	472	222	120	38	345	97	401	179

Table II shows the results of a single test with KCl. No germination was obtained at 0.01M, and the average length of the tubes was greatly reduced at 0.002M and 0.001M. At 0.0002M the growth was stronger than in the check culture. In some further experiments in which KCl was employed, no germination was obtained at 0.01M in three tests; at 0.0066M, germination was inhibited in two trials; and a few tubes were produced in a third test. No tubes were produced in two tests at 0.004M, a few in one trial, and high germination was shown in two tests.

The only other salt of a monovalent cation used was LiCl. No germination was obtained at 0.02M in one trial, nor at 0.01M in six trials, nor at 0.0066M in three trials. At 0.004M five tests were

made; no germination was obtained in two cases, a few tubes were produced in one test, and about 50 per cent germination was obtained in each of the other two trials.

TABLE II

LENGTH IN MICRONS OF POLLEN TUBES GROWN IN SUCROSE CULTURES CONTAINING KCl AS STATED

CHECK		0.01M KCl		0.002M KCl		0.001M KCl		0.0002M KCl		$CaCl_2$	
No.	Length	No.	Length	No.	Length	No.	Length	No.	Length	No.	Length
115	234	0	0	57	73	69	86	92	327	111	389

The results concerning the effect of the salts of the monovalent cations Na, K, and Li on growth, taken together with those relating to the concentrations that will just prevent germination, show that the substances in question do not differ greatly in their toxicities.

Toxicity of salts of magnesium, barium, and strontium

In a preliminary test with $MgCl_2$ it was found that germination is entirely prevented at 0.01M, 0.002M, and 0.001M. At 0.0002M, however, growth was obtained equal to that in the check culture. In further experiments designed to determine the concentration of $MgCl_2$ which would just prevent germination, no tubes were produced in two tests at 0.00066M, a very few in one trial, while in four other tests the percentages of germinated grains ranged from 10 to 85.

TABLE III

LENGTH IN MICRONS OF POLLEN TUBES GROWN IN SUCROSE CULTURES CONTAINING $SrCl_2$ AS STATED

CHECK		0.01M $SrCl_2$		0.002M $SrCl_2$		0.001M $SrCl_2$	
No.	Length	No.	Length	No.	Length	No.	Length
42	381	0	0	69	81	69	496

With $BaCl_2$ no germination was obtained at 0.01M, 0.002M, and 0.001M, with the exception of a few very short tubes produced in one of the six trials made at the last concentration. At 0.00066M no germination was obtained in three trials, about 10 per cent in three

tests, and about 15 per cent in a seventh trial. Less than 5 per cent germination resulted in two tests at 0.0004M, but in the remaining four trials at this concentration germination was high. At 0.0002M little or no toxicity was in evidence. On the basis of these experiments it is concluded that the chlorides of the bivalent cations Mg and Ba are roughly fifteen times as toxic as the corresponding salts of the monovalent cations Na, K, and Li.

That the nature of the cation as well as its valency must be considered, however, is shown by the results with $SrCl_2$, and those with $CaCl_2$ to be presented in the section following. While no germination was obtained with $SrCl_2$ in concentrations of 0.01M and 0.002M, the results at 0.001M and 0.00066M show it to be appreciably less toxic than $MgCl_2$ and $BaCl_2$. Two tests were made with 0.001M solutions; in one no germination was obtained, but in the other most of the grains produced tubes. At 0.00066M over 60 per cent germination was obtained in each of two trials. Table III shows the amount of growth of sweet pea pollen tubes in 0.002M and 0.001M solutions of $SrCl_2$ as compared with the check culture, which contained 15 per cent sucrose only. It will be noted that at 0.001M the average length of the tubes exceeds that in the salt free medium. The toxicity at 0.002M, however, is marked.

Influence of calcium salts

After having tried several single neutral salts which were invariably found to be highly injurious, it was interesting to discover that $CaCl_2$ in corresponding concentrations promoted growth in a very marked degree. The average lengths of sweet pea pollen tubes in cane sugar media containing calcium chloride in amounts ranging from 0.05M to 0.002M are given in table IV. Increases in growth are shown in 0.02M, 0.01M, and 0.002M. Where $CaCl_2$ is present in a concentration of 0.01M this increase amounts to nearly 50 per cent. At 0.05M the average for two tests shows somewhat less growth than the check, but the toxicity is not great. A further experiment showed that in the presence of 0.2M or 0.1M $CaCl_2$ no germination occurs.

In table V the results are given of an experiment to determine the relative growth-promoting powers of three different calcium salts

at two concentrations, 0.01M and 0.002M. It will be observed that the increases in growth in the cultures containing the calcium salts in concentrations of 0.01M are very much greater than those obtained in the preceding experiment. This is due both to a lesser growth in the check cultures and a greater absolute growth in the media containing the calcium salts. Inspection of the separate

TABLE IV

LENGTH IN MICRONS OF POLLEN TUBES GROWN IN SUCROSE CULTURES CONTAINING $CaCl_2$ AS STATED

TEST	CHECK		0.05M $CaCl_2$		0.02M $CaCl_2$		0.01M $CaCl_2$		0.002M $CaCl_2$	
	No.	Length	No.	Length	No.	Length	No.	Length	No.	Length
1	90	232	.....	.....	.....	.....	108	257	90	269
2	96	331	95	251	95	365	92	625	96	451
3	48	144	72	152	72	264	72	243	48	312
Average	234	254	167	209	167	321	272	378	234	353

TABLE V

LENGTH IN MICRONS OF POLLEN TUBES GROWN IN SUCROSE CULTURES CONTAINING $CaCl_2$, $Ca(NO_3)_2$, OR $CaSO_4$ AS STATED

TEST	CHECK		0.01M $CaCl_2$		0.002M $CaCl_2$		0.01M $Ca(NO_3)_2$		0.002M $Ca(NO_3)_2$		0.01M $CaSO_4$		0.002M $CaSO_4$	
	No.	Len.	No.	Len.	No.	Len.	No.	Len.	No.	Len.	No.	Len.	No.	Len.
1	103	49	31	689	65	118	35	973	62	106	55	984	56	398
2	72	154	38	767	72	208	48	778	65	325	72	446	38	1003
3	72	156	44	986	72	418	33	910	61	529	20	1063	24	1215
Average	247	97	113	831	209	276	116	875	188	319	147	731	118	759

values obtained for each medium in each of the three tests will afford some idea of the variability encountered in such experiments as these. The main facts, however, stand out rather clearly. At 0.01M, $CaCl_2$, $Ca(NO_3)_2$, and $CaSO_4$ give average amounts of growth of similar magnitudes. The lengths of the pollen tubes in the 0.002M culture of $CaCl_2$ and $Ca(NO_3)_2$ are likewise in fairly close agreement. With 0.002M $CaSO_4$, however, the average length of the tubes is much greater. Since the three tests agree in this regard,

the results are possibly significant, but the point should be tested further.

The data relating to the effect of the chlorides of sodium and potassium as compared with the same calcium salt indicate that in the phenomena under discussion the cation plays the dominant rôle. As a whole the results of the experiments with different calcium salts support this view, although the evidence is not unequivocal, as shown by the 0.002M $CaSO_4$ cultures.

Protective action of calcium in salt mixtures

Having demonstrated that calcium salts in sucrose cultures showed no toxicity to sweet pea pollen except when present in concentrations greater than 0.04M, and that in more dilute solutions their effect on growth was decidedly salutary, attempts were made to determine whether they exerted any protective effect in the presence of other salts known to be injurious when used singly.

TABLE VI

LENGTH IN MICRONS OF POLLEN TUBES GROWN IN SUCROSE CULTURES CONTAINING NaCl OR $CaCl_2$ OR MIXTURES OF THESE SALTS

TEST	CHECK		0.01M NaCl		0.04M $CaCl_2$		0.01M $CaCl_2$		0.01M NaCl+ 0.04M $CaCl_2$		0.01M NaCl+ 0.01M $CaCl_2$	
	No.	Len.	No.	Len.	No.	Len.	No.	Len.	No.	Len.	No.	Len.
1.....	105	161	0	0	102	179	96	283	84	303	105	389
2.....	72	118	0	0	72	185	72	659	72	270	72	375
3.....	48	109	0	0	72	229	72	521	72	188	72	452
4.....	72	306	.....	.....	69	98	95	571	94	186	96	408
Average.	297	177	0	0	315	174	335	497	322	236	345	405

The results of experiments with mixtures of NaCl and $CaCl_2$ are given in table VI. In this series of tests, cultures containing the respective single salts in corresponding concentrations were also included, except in Test 4, where the 0.01M NaCl member was lacking. The results are illuminating. No germination was obtained in any of the cultures containing 0.01M NaCl alone. On the average, the growth in the presence of 0.04M $CaCl_2$ was nearly equal to that in the salt-free check cultures. In the media containing 0.01M NaCl+0.04M $CaCl_2$ the pollen tubes averaged over 30 per cent

longer than the check culture. Not only is the toxic effect of the NaCl prevented, but the growth exceeds that in the 0.04M $CaCl_2$ culture by about 35 per cent. That is, the toxicity of both salts is reduced when they are combined. The injurious effect of 0.01M NaCl is also overcome when the amount of $CaCl_2$ is reduced to 0.01M. The average length of the tubes with this combination of salts, however, is nearly 20 per cent less than when 0.01M $CaCl_2$ alone is present.

TABLE VII

LENGTH IN MICRONS OF POLLEN TUBES IN SUCROSE CULTURES CONTAINING 0.02M KCl AND $CaCl_2$ AS STATED

Test	Check		0 $CaCl_2$		0.001M $CaCl_2$		0.002M $CaCl_2$		0.01M $CaCl_2$		0.02M $CaCl_2$		0.04M $CaCl_2$	
	No.	Len.	No.	Len.	No.	Len.	No.	Len.	No.	Len.	No.	Len.	No.	Len.
1	45	63	0	0	0	0	24	66	60	402	30	278	25	96
2	53	86	0	0	45	91	62	99	46	564	49	589	70	87
3	70	81	78	50	59	67	93	90	45	614	36	560	65	93
4	72	119	0	0	56	47	72	97	35	617	34	736	0	0
5	52	74	37	81	38	47	64	117	72	391	48	464	72	132
6	70	332	0	0	72	94	72	119	72	255	72	544	72	107
Average	362	134	115	60	270	71	387	101	330	442	269	535	304	105

In two further experiments attempts were made to determine the amounts of $CaCl_2$ necessary to render KCl harmless when the latter salt was present in concentrations of 0.02M and 0.04M respectively. Table VII shows that when 0.02M KCl cultures are made 0.01M and 0.02M for $CaCl_2$, not only is the injurious effect of the former substance overcome, but the growth is three to four times as great as in the salt free check medium.

Three tests were made with the same concentrations of $CaCl_2$ in cultures containing 0.04M KCl. The results are presented in table VIII. Relatively long pollen tubes were produced in the check culture in Test 2, bringing the average for that medium considerably above the value for the corresponding medium in the previous experiment. It will be noted that when the amount of $CaCl_2$ present is reduced to 0.001M it is no longer effective in overcoming the toxicity of 0.04M KCl. In the cultures containing 0.04M KCl+0.01M $CaCl_2$ the average growth exceeds that in the

check medium. When the amount of $CaCl_2$ is reduced to 0.002M, however, the length of the tubes is only about one-third that in the culture containing sucrose only. In the 0.04M KCl+0.02M $CaCl_2$ cultures, contrary to expectation, the toxicity is rather marked.

TABLE VIII

LENGTH IN MICRONS OF POLLEN TUBES IN SUCROSE CULTURES CONTAINING 0.04M KCl AND $CaCl_2$ AS STATED

TEST	CHECK		0		0.001M $CaCl_2$		0.002M $CaCl_2$		0.01M $CaCl_2$		0.02M $CaCl_2$		0.04M $CaCl_2$	
	No.	Len.	No.	Len.	No.	Len.	No.	Len.	No.	Len.	No.	Len.	No.	Len.
1.....	39	137	0	0	0	0	66	75	72	312	72	135	57	65
2.....	72	536	0	0	0	0	48	65	72	587	72	219	72	85
3.....	36	234	0	0	0	0	42	180	48	587	54	325	72	78
Average...	147	356	0	0	0	0	156	100	192	484	198	218	201	77

In view of the variability in the results of the several tests, additional data will have to be obtained before definite conclusions can be drawn regarding the quantitative aspects of these relations.

Effects of salts on pollen of Nicotiana sanderae

A study was made of the effects of salts on the pollen of *Nicotiana sanderae*, in an attempt to determine to what extent the findings in regard to sweet pea pollen might have general validity. The available *Nicotiana* pollen, however, was not well suited to the purpose. Many of the grains were defective and evidently incapable of producing tubes, and possibly an appreciable percentage of those which appeared morphologically complete were likewise nongerminable. Since no other species was at hand at the time, however, the *Nicotiana* pollen was employed. The values given for germination percentages are only approximate, being based upon rough counts under the microscope of two or three fields in each culture.

The results were highly variable, but a sufficient number of tests were made to establish certain facts rather definitely. LiCl, NaCl, and KCl proved injurious to *Nicotiana* pollen in approximately the same degree as had been found for the sweet pea. There were

greater differences, however, between the effects of the three different salts. LiCl was the most toxic, preventing germination entirely at 0.0066M; NaCl was equally toxic at 0.013M to 0.01M; while KCl permitted sporadic germination even at 0.02M.

It was interesting to find that the reactions of *Nicotiana* pollen to $CaCl_2$ and $MgCl_2$ were quite different from those of the sweet pea. It will be recalled that $CaCl_2$ exerted a markedly stimulating effect upon the pollen tubes of the latter form. In the case of *Nicotiana*, while 0.01M $CaCl_2$ gave 50–70 per cent germination in different tests, vigorous tubes were never developed. The toxic effect on the pollen tubes was marked, even in 0.002M $CaCl_2$ cultures. On the other hand, high germination and strong growth were repeatedly obtained in cultures containing 0.002M $MgCl_2$. The percentage of germination was not lowered when the concentration of $MgCl_2$ was increased to 0.01M, although the average length of the pollen tubes was less. The similarity of the effect of $MgCl_2$ on *Nicotiana* and $CaCl_2$ on sweet pea may mean that their action in the two cases is analogous.

$BaCl_2$, as in the case of the sweet pea, proved very toxic to *Nicotiana* pollen, inhibiting germination entirely at 0.0005M, and permitting it only in very meager amounts at 0.0004M. $SrCl_2$ was somewhat less injurious. No germination was obtained at 0.002M in three tests, or at 0.001M in three tests. In three other trials at the latter concentration, however, about 5 per cent germination was obtained in two cases, and 50 per cent in the third. In four cultures containing this salt in a concentration of 0.0005M, the percentages of germination ranged from 10 to 80, but the tubes produced were all very short.

$AlCl_3$ and $Ce(NO_3)_3$, were found to be extremely toxic to *Nicotiana* pollen. Four tests with the former salt and five with the latter showed complete suppression of germination at 0.0004M. At 0.0002M, $Ce(NO_3)_3$ gave no germination in three trials and about 50 per cent in one trial. No germination was obtained at 0.0002M in three tests with $AlCl_3$; in three other cases about 10 per cent was observed; and in three further tests gave from 25 to 50 per cent.

Several trials were made also with salts of the heavy metals Cu, Ni, Zn, Mn, and Fe. $CuCl_2$, $CuSO_4$, and $NiCl_2$ completely

inhibited germination at 0.0004M. Very sparse germination was obtained in one-half the trials at 0.0002M, and none in the remainder. While highly toxic at 0.0005M, $ZnCl_2$ exerted a markedly stimulating effect at 0.000266M and 0.0002M. At these concentrations growth was usually stronger than in the salt-free check medium. This observation is in accordance with the results others have obtained with various plants grown in dilute solutions of zinc salts. $MnCl_2$ also was found to stimulate growth very appreciably at 0.002M and 0.001M. The pollen tubes produced in the manganese cultures were regularly coiled in a characteristic spiral fashion. At concentrations of 0.0004M and 0.0002M, $FeCl_2$ gave sporadic germination and usually very short tubes.

Discussion and summary

The most striking feature of these results is the extreme sensitiveness shown by pollen tubes to salts. It has been demonstrated that NaCl, one of the least toxic of the neutral salts tried, excepting $CaCl_2$, added to a sucrose solution in a concentration of 0.0002M, or about 11 parts per million, reduces the growth of sweet pea pollen tubes 15 per cent. A concentration of 0.01M NaCl inhibits germination entirely. $MgCl_2$ and $BaCl_2$ are roughly fifteen times as toxic as NaCl. *Nicotiana* pollen is likewise highly susceptible to the injurious effects of single salts, although in certain cases its reaction differs considerably in degree from that of the sweet pea. Possibly this hypersensitiveness is due to very rapid penetration of the protoplasts by the substances in question.

It has been established that in the presence of calcium salts in concentrations ranging from 0.02M to 0.002M, the growth of sweet pea pollen tubes is markedly enhanced. $MgCl_2$ has a similar action on *Nicotiana*. So far as the writer has been able to detect by microchemical means, these salts do not affect the wall of the pollen tube; presumably they act on the protoplast itself. In the light of recent experimentation (**16**) it is not unlikely that the beneficial influence of these salts is related to changes effected in the permeability of the protoplasts. Evidence has been obtained from other forms that the maintenance of normal permeability, an important condition in vital phenomena, is intimately related to the salt content of the

surrounding solution. Possibly in pure sucrose cultures exosmosis is a limiting factor in pollen tube growth; the addition of salts of calcium or magnesium may favor development by retarding or preventing this outward diffusion.

In the protective action exhibited by calcium in solutions containing another salt known to be highly injurious when present alone, we undoubtedly have a well marked case of antagonism. This interpretation is in harmony with the writer's suggestion that the dominant rôle of calcium is in preserving normal permeability, and that it does not act as a true nutrient. The necessity of balancing electrolytes in culture media has been widely recognized, but this phase of pollen physiology is singularly barren of facts. Much improvement might be made in cultures for pollen with a fuller knowledge of these relations.

It is by no means improbable that the pollen tube is independent of external sources of salts, so far as these latter are required as nutritive elements in the narrow sense of the term. The present results would indicate that electrolytes present in the style may play an important rôle, however, in regulating the diffusion of substances through the surface layer of the protoplast of the pollen tube. EAST (7) found that when compatible and incompatible pollen tubes are growing in a single pistil in self-sterile *Nicotiana*, the former group only functions in reproduction. This shows, as EAST has pointed out, that the reaction between pollen tube and surrounding cells must be local. Each sort of microgametophyte maintains its distinctive character in a common environment. Whatever may be the nature of the reaction set up between the compatible tubes and the conducting tissue, enabling the former to complete their development, the adjacent incompatible tubes do not benefit thereby. This difference in the chemical processes of compatible and incompatible tubes is made possible by the existence of semi-permeable membranes. It is not unreasonable to assume that, so far as pollen tube growth is concerned, the chief function of the salts in the style is the preservation of the semi-permeable nature of the protoplasmic membranes.

In connection with these findings on the effects of salts, it is interesting to note certain facts regarding the mode of growth of the

pollen tube in the style. PATON (**17**) has demonstrated that pollen contains pectinase. It is the secretion of this enzyme, presumably, that enables the pollen tube to pursue its intercellular growth, since the middle lamella, as the work of MANGIN (**12**) shows, consists of pectose in combination with inorganic bases, principally calcium. In the digestion of the middle lamella these bases would be set free. In view of the pronounced effect of calcium upon the growth of the pollen tube of sweet pea, the digestion of the intercellular substance in the style may have a significance in the development of the male gametophyte not hitherto suspected.

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ANATOMY OF CHEILANTHES TENUIFOLIA

MAY M. WILLIAMS

(WITH PLATES VIII–X)

Introduction

Cheilanthes tenuifolia Swartz grows in all the dry parts of New South Wales, and is a common member of the xerophilous flora. It has adapted itself to situations in which it may be absolutely dry for several months during each year, but as soon as rain comes it commences to grow immediately. The material for the present investigation was collected at Kuring-gai Chase and Picton, New South Wales.

The fern is not very large. It consists of a subterranean dorsiventral rhizome which occasionally branches and attains a considerable length. Stiff erect fronds rise above the surface of the soil here and there, usually in clumps. This is due to the fact that the fronds grow out from the rhizome much more quickly than the rhizome itself grows. Meanwhile, the fronds along the older parts of the rhizome die, so that there remains a clump of leaves around the apex. There is a very efficient root system growing out from the rhizome, the main roots branching profusely and giving rise to a very extensive secondary root system. The roots are dark brown, and very thin and wiry.

The older portions of the rhizome are covered by the leaf bases of the older leaves, and numerous scales. The young leaf shows circinate vernation, and in the early stages is covered with scales. These, however, fall off as the leaf expands.

The petiole of the leaf is about 6 inches long, thin and wiry, black, and polished. It is quite smooth for the greater part of its length, but toward the base a few scales occur. Midway along the length of the petiole pinnae arise. The frond is tripinnatifid, the pinnae being arranged in numerous alternate pairs. HOOKER and BAKER (**7**) regarded them as being arranged in numerous opposite pairs, but the writer's observations did not confirm this.

The pinnae are arranged very close to one another and appear to be opposite, but the vascular bundles come off alternately. The rachis is polished like the petiole, and is the same color. Both surfaces of the pinnae are green and naked, and the margins are inrolled to form an indusium. The veins arise from a central midrib, are forked, and bear sori on their ends.

The material was fixed in the field in a chromo-acetic solution. After fixation the material was thoroughly washed, dehydrated, and passed in the usual way into paraffin. Sections were then made of the various parts, the thickness varying from 3 μ to 10 μ, according to the part of the plant which was being cut. The sections were then stained by one of a number of straining pocesses, but Fleming's triple stain proved by far the most satisfactory for every part.

Investigation

Rhizome

The rhizome is very long and thin, covered for the greater part of its length by the leaf bases and scales. It is dorsiventral, with stiff erect fronds and thin wiry roots. Bower (1) considers that creeping rhizomes are probably derivative. "That large leaved forms would be mechanically unstable is obvious, especially when the stem is thin and the internodes of appreciable length. There is an inherent probability that such axes should become oblique or prone." The rhizome occasionally branches, these branches always bearing the same structure as the main axis. From the condition seen in fig. 1, it would appear that branching is dichotomous, although monopodial branching is of common occurrence among the Polypodiaceae as a whole.

Numerous young leaves arise around the apex. These show circinate vernation, and curl around the apex of the rhizome, thus forming some protection for the latter.

A section of the apex of the stem shows that it becomes hidden by scales, which grow out from the epidermis, thus forming a still more efficient protection for the apex. The termination of the stem is broad and flat, and from this the apex arises rather abruptly as a small cone, terminated by a tetrahedral apical cell (figs. 1, 2). Segments are cut off from two cutting faces, and from these are

formed the older tissues of the rhizome, also the roots and leaves. These segments, cut off parallel to the two cutting faces, are again divided by a wall perpendicular to the long axis of the cell, forming an outer and an inner cell. The former is again divided, so that there are two outer and one inner cell formed from each segment. The inner cell goes to form the vascular cylinder, the outer cells form the cortex and epidermis. The vascular tissue is delimited from the ground tissue to a point very near the apex.

SACHS states that creeping stems with a bilateral structure have a two-sided apical cell, while the erect ascending stems with rosettes and leaves radiating in every direction have a three-sided pyramidal apical cell. KLEIN, however, later disproved this generalization.

More recently BOWER, as a result of his observations in the meristems of a large number of ferns, has stated that in the leptosporangiate ferns, whether radial or erect, the apex is occupied by a three-sided pyramidal apical cell. This generalization, however, is not without its exceptions among dorsiventral forms such as *Pteris aquilina*, *Salvinia*, and *Azolla*, in which a two-sided apical cell is present. *Cheilanthes tenuifolia* is another exception to this rule, for here also a two-sided apical cell is present.

The stem anatomy of the various species of *Cheilanthes* is of interest, in that it contains both solenostelic and dictyostelic types, and forms which are transitional between these two. A section of the rhizome of *C. tenuifolia* shows it to be a dictyostelic type, according to GWYNNE-VAUGHAN'S (**5**) definition, that is, the continuity of the vascular cylinder is interrupted at more than one point by overlapping leaf gaps (fig. 4). The cortex of the stem becomes transformed into sclerenchyma, and from the outer layers of this scales arise which protect the rhizome. The pith also becomes sclerized, so that sections show a mass of sclerenchyma surrounding a varying number of meristeles.

The detailed anatomy of the stelar tissues furnishes results of considerable interest (fig. 5). The center of the meristele is occupied by xylem accompanied by wood parenchyma. The xylem is composed wholly of scalariform tracheids; in no instance were there any spiral or annular tracheids corresponding to the protoxylem. Sections were made of the apical region and thoroughly examined, but

even here no trace of protoxylem was observed, the first tracheids to be lignified being large scalariform elements. This condition was also observed by MARSH (**9**), who noted that there was no protoxylem present in the stems of *Cheilanthes Fendleri*, *C. gracillima*, *Pellaea andromedaefolia*, and other xerophilous forms.

This absence of protoxylem in the vascular bundles of stems is to be found in various other members of the Filicales. BOWER (**2**) states that in *Lophosoria* "the tracheids are of uniform character, and, as is often the case in the Cyatheaceae, there is no protoxylem. Very soon after leaving the axis, protoxylem groups make their appearance in the vascular strand which had hitherto been without them." GWYNNE-VAUGHAN (**6**) states:

> There are a large number of ferns in which the protoxylems of the petiolar bundles die out toward the base of the trace, disappearing entirely before or immediately after its insertion upon the stele of the stem. No definitely localized protoxylem strands are to be found in the stem of these ferns, nor are there any spiral or annular tracheids present.

He maintains that the first formed scalariform elements of the xylem, which are usually smaller than those formed later on, in these forms, may be considered to represent protoxylem elements. The walls of the scalariform tracheids have a well defined beaded appearance (fig. 7).

It was observed that with the tracheary tissue is mingled more or less wood parenchyma. MARSH concludes that there is a close relation between the stelar structure and the amount and arrangement of wood present within the stele. He observed that in perfectly solenostelic types of *Cheilanthes*, such as *C. Fendleri*, and in types which scarcely depart from this, such as *C. gracillima*, the xylem forms a continuous band of tracheids, not broken by parenchyma. *C. lanuginosa*, which is intermediate between these types and large stemmed dictyosteles, has a wider band of tracheids slightly broken by parenchyma. *C. persica*, a dictyostelic form, has a broader band of wood, in which considerable parenchyma occurs. *C. tenuifolia*, therefore, gives additional support to these statements.

Separating the xylem and phloem are a few layers of parenchyma, the "vasal parenchym" of STRASBURGER. The phloem is

both external and internal, the amount of external being about equal to the amount of internal phloem; nevertheless there is very little phloem present in the stem. The total number of sieve tubes in no instance is large. There is no differentiation into metaphloem and protophloem; the elements are narrow and arranged in a single row on either side of the xylem ring. The phloem ring is not continuous, but is interrupted at the leaf gap (fig. 6). MARSH observed that a similar condition occurred in other species of *Cheilanthes*.

In *C. tenuifolia*, surrounding the whole meristele is a layer of brown cells, differing from the outer sclerenchyma, which constitute the endodermis. Separating the phloem and endodermis are three, four, or perhaps five layers of fairly large cells containing considerable granular matter. The only interpretation of these cells is that they represent the pericycle, which in this instance, therefore, is many layered.

CAMPBELL (3) mentions that in certain types of Filices the pericycle may be double, and usually contains considerable starch. According to STRASBURGER these cells do not constitute a true pericycle, but belong to the cortex. They are the sister cells of the endodermis, which is thus not the innermost cortical layer, but the next but one. This many layered pericycle probably acts as a storage system for the reserve foods of the plant. By this means, as soon as conditions are again suitable for growth, the food materials are available.

The root trace is given off from the outer margin of the stele and passes through the cortex with the minimum of disturbance.

The leaf trace departs from the vascular cylinder as a single *C*-shaped bundle, and passes through the cortex of the stem in the same condition, associated with sclerenchyma from the pith. The leaf trace is given off from the center of the meristele. This single arched condition of the leaf trace observed in *C. tenuifolia* is of considerable interest, since it indicates that although the stem possesses a dictyostelic structure, it is a primitive member of these types, and dictyostely has just evolved within the group. The type of leaf trace which is present is characteristic of solenostelic types.

GWYNNE-VAUGHAN (6), speaking of the evolution of the dictyostele, states that "an examination of transitional types makes it

clear that the dictyostely of the Cyatheaceae and Polypodiaceae is primarily due simply and solely to the overlapping of the leaf gaps in the solenostele."

Briefly summarizing his conclusions, TANSLEY (11) states as follows:

> The leaf trace of the petiolar vascular system of ferns, though correlated in a general way with the structure of the stem stele, has a semi-independent course of origin which it is necessary to consider separately. GWYNNE-VAUGHAN has done good service in calling attention to the fact that the arched type of leaf trace, or some clearly derived form, is to be found in the vast majority of ferns, and is certainly to be regarded as typical. As we pass from protostelic to solenostelic forms, we find the leaf trace taking on a corresponding expansion. BOODLE has attributed the origin of the solenostele, with its increased diameter, to the necessity for accommodating the insertion of broader leaf traces, thus implicitly recognizing the fact, which seems to be indisputable, that the leaf trace leads and the stele follows in the course of evolution.

The results of the present investigation do not appear to confirm the latter part of this statement, since, although the leaf trace is still in the simple condition typical for solenostelic types, the stem has advanced and a perfect dictyostele has been produced. The general rule is that dictyostely is accompanied by the division of the single strand into many.

BOWER (1) states:

> All the more primitive types of ferns . . . . have a more or less horse-shoe shaped trace, but the modern Marattiaceous, and the bulk of the Polypodiaceous forms have a trace composed of many strands; these are however arranged in a series corresponding to the outline of the undivided trace. These facts indicate, with no possible uncertainty, that there has been disintegration of the leaf trace by fission; it finds its origin in the enlarged upper region of the leaf, and has been phyletically progressive from a region lying towards the base. Thus in several distinct phyla it is shown that a progressive disintegration of the axial stele has followed the expansion of the axis.

It seems clear that the former part of TANSLEY'S statement is the more correct, namely, that the leaf stele has a semi-independent course of origin, which it is necessary to consider separately.

Another feature of interest with regard to the leaf trace is that its separation from the stem stele is not effected synchronously on both sides, one side separating before the other. BOWER (2) has observed

that this also occurs in *Lophosoria*, a solenostelic type of the Cyatheaceae, and claims that it is usual in ferns with a creeping axis.

A study of the stem anatomy of *C. tenuifolia* places it among the phyllosiphonic type of JEFFREY'S (8) classification, that is, those types in which the leaves are given off with profound disturbance of the cauline system.

LEAF

The leaf arises from the segments produced by the apical cell of the stem. It first becomes evident as a small conical emergence, at the apex of which is a wedge-shaped apical cell. Segments are cut off parallel to the two cutting faces of this cell. Each segment becomes divided by a transverse wall into an outer and an inner cell. The outer cell becomes further divided by a longitudinal wall. The superficial cells thus produced may give rise to pinnae, growth being due in this instance to a marginal cell. The growth of the leaf is always more rapid on one side than it is on the other, so that the apex tends to become curved inward. This uneven growth gives rise to the circinate vernation of the young leaf, and thus the young axis is protected (fig. 8). BOWER asserts that in the Polypodiaceae the young apical cell is three-sided, but later it becomes two-sided. *C. tenuifolia* is an exception to this statement, for in every instance the apical cell was two-sided even in the young stages.

Petiole

Fig. 9 represents a section typical of the petiole. It will be observed that the petiolar stele forms a single strand, and remains in this condition along its whole length except where branches are given off to the pinnae. This single strand is surrounded by cortex and epidermis, which, as the leaf grows, becomes transformed into sclerenchyma, thus giving the mechanical support necessary, since the petiole itself is thin and wiry. The contour of the petiole coincides mainly with that of the vascular strand. The latter is chiefly composed of xylem, consisting of both protoxylem and metaxylem. When the leaf trace becomes separated from the stem stele it is a simple, adaxially curved, anarch structure, and protoxylem groups make their appearance within it. The protoxylem elements are composed of spiral and annular tracheids. There are

three protoxylem groups occurring in the petiole, two of which are endarch in position, the other in a few instances medianly attached and exarch, but in sections made of the older petiole, medianly placed but detached.

Fig. 10 indicates a condition which seems to be the earliest occurring in the petiole after the simple, adaxially curved, anarch leaf trace, and is found at the base of the petiole of the young leaf. Here are represented three protoxylem groups, two of which are endarch and lateral. The third medianly situated exarch group seems to have divided the metaxylem elements, for the greater part unlignified as yet, into two distinct groups separated by parenchyma and the protoxylem group. Fig. 11 represents the condition found in the older stem. There the metaxylem elements have become grouped into a single continuous band, and the third protoxylem group has become detached from the remainder of the xylem elements.

The most feasible explanation of the manner in which these conditions have been attained is to be obtained by a careful study of the types already described by MARSH. He maintains that in all the species which he investigated there is at the base a simple, adaxially curved, anarch leaf trace, but as the petiole is followed upward the changes which occur vary considerably. In the simplest expression three endarch protoxylem groups are developed in this simple bundle; the median group migrates and takes an exarch position, and finally becomes detached altogether. The various stages which MARSH has described as occurring in *C. Fendleri* are not all to be observed in *C. tenuifolia*, but this may be due to secondary simplicity of the petiolar structure, resulting in the dropping out of various types at the base.

MARSH claims that these facts admit of only one explanation, that the base of the leaf preserves primitive characters. This is also the position taken by SINNOTT (10), who has pointed out that the leaf traces of all ferns, with a few exceptions, can be derived from a triarch-mesarch leaf trace, and that the nearest approach to this condition is usually to be found at the leaf base. The mesarch protoxylems in the course of evolution have gradually become endarch, and in many forms all trace of mesarchy is lost,

even at the leaf base. This is the condition found at the leaf base of *C. Fendleri*, and it is only by assuming that the acropetal succession recapitulates the evolutionary succession that we can reasonably bring the peculiar petiole of *C. Fendleri* into line with the normal filicinean leaf trace.

The actual cause of the exarchy and detachment of the median protoxylem group is so far unexplained. It has been claimed by many investigators that it is at the base of the petiole that any change will occur first, since it is here (as the leaf in the evolutionary ascent enlarges) that the greatest demand for an increased water supply will be most keenly felt. Fig. 10 seems to indicate that the single strand of the petiole is becoming double, but the separation into two bundles has not actually taken place. A break in the protoxylem group, which forms the sole link between the two distinct xylem masses, would mean that two distinct xylem strands would exist, each with two protoxylem groups, and in many instances this was seen to have occurred. The phloem ring surrounding the xylem in a few instances is broken, but the endodermis and pericycle are still continuous. It would seem that this serves to indicate how a doubling of the petiolar bundle may have occurred. The condition found higher in the petiole is quite in harmony with this; the demand for water is not felt so keenly here as lower down. The metaxylem elements have become continuous, and the median protoxylem group has become detached.

The condition in which there exists a double trace at the base, which unites to form a single strand at a higher level, is to be observed in other genera, such as *Onoclea sensibilis* and *Diacalpe*.

The metaxylem is composed of scalariform tracheids of the same nature as those occurring in the stem. Here also there are a few layers of parenchyma separating the xylem and phloem. The phloem is poorly developed, consisting of a single row of sieve tubes surrounding the xylem, but absent from the sides of the bundle and also from the vicinity of the median protoxylem group (figs. 12, 13). The same explanation may be applied to the poor development of the phloem in the petiole, as was mentioned in the case of the rhizome. The vascular strand may be regarded as concentric, the absence of phloem on the sides being due to reduction.

Surrounding the phloem are two or three layers of parenchymatous cells containing dense granular cytoplasm. These, as in the rhizome, might be interpreted as representing a many layered pericycle, the cells storing quantities of reserve food material. This is surrounded by the endodermis of the same nature as that of the rhizome. The cortex is sclerenchymatous.

When the petiole reaches the level of the pinnae, the vascular strand gives off branches which enter the rachis, each rachis containing a single strand of the same nature as that of the petiole. This again branches in the rachis and supplies the vascular strands to the pinnules.

Pinnules

The veins of the pinnules always show dichotomous branching, and on the ends of these forked veins sori are borne. No hairs or scales are present, although they occur in the bud. The minute anatomy of the pinnule is represented in fig. 14. The upper epidermis is composed of large cells, protected by a cuticle. The stomata are confined to the lower epidermis, and are protected by the inrolled margins of the leaf alone. In this respect *C. tenuifolia* is similar to the xerophilous fern *Pellaea andromedaefolia*, described by MARSH. Other xerophilous species of *Cheilanthes* have the stomata protected by the inrolled margins and a covering of scales, or by hairs. A surface section of the lower epidermis (fig. 18) indicates the nature of the stomata. The guard cells contain chlorophyll, granular cytoplasm, and a large nucleus. The cells of the lower epidermis have a convoluted outline.

The palisade tissue is well developed, and is two or three cell layers deep. The cells have the chromatophores distributed around the margins of their walls. The lacunar tissue is reduced, the cells of which may contain chromatophores.

The vascular system of the lamina is supplied by veins, which run out obliquely from the midrib of the pinna. The vascular bundles have a well marked endodermis (fig. 16), the cells of which are large, colorless, and thin walled. Below this is a single (sometimes a double) layer of cells of identically the same nature as those already described as occurring beneath the endodermis of the stem and petiole. These cells may be regarded as part of the pericycle

in which reserve food is stored until it is required by the plant. A single layer of sieve tubes representing the phloem surrounds the xylem, although at intervals its continuity is interrupted by parenchymatous cells. The vascular supply to the developing sporangia is represented by a vascular commissure which runs below the whole area of the sorus.

These features indicate that in the leaf there are well marked adaptations both in texture and in the minute anatomy to the habitat of the fern.

Root

The root system always arises in connection with the bases of the leaves, and consists of a number of delicate fibrils branching freely, producing a very extensive root system. The new branches arise in two lines corresponding to the ends of the xylem plate in the diarch bundle. A longitudinal section of the rhizome, such as is diagrammatically represented in fig. 17, and a portion of which is shown in detail in fig. 18, indicates the endogenous origin of the root. Van Tieghem, who has made an exhaustive study of the subject, states that they always arise from an endodermal cell.

The root apex is protected by a root cap, and also in its younger stages by the scales of the rhizome. This latter condition is represented in fig. 19. The root is formed by the cutting off of segments from a three-sided pyramidal apical cell (figs. 17–20). From the segments cut off parallel to the two lateral faces the tissues of the root arise. Segments cut off parallel to the remaining face are formative of the root tissue in part, but mainly of the root cap. The outer cells of the root cap are continually being worn away, and as continually being renewed by division of segments from the apical cell (fig. 20). The xylem first makes its appearance as two groups of tissue which appear simultaneously as the foci of the section (fig. 21). The elements are very small and have spiral and annular thickening on their walls. These protoxylem groups are differentiated long before any of the other elements. The remainder of the tissue at this stage is thin walled, and shows no marked characters, excepting that the endodermis even at this stage is well defined. The cortex also is composed of thin walled cells.

A curious feature of this form is that no definite root hairs are developed. Some of the epidermal cells may show short protuber-

ances, but there do not seem to be any normal, well defined root hairs. In the absence of these it follows that absorption must take place in one of two ways; either mycorrhiza may be present or the epidermal cells themselves may be especially adapted to absorb soil water by osmosis. In the absence of the former, it follows that the latter must take place. This perhaps may be a special adaptation under which the plant grows; root hairs would readily perish in the dry soil. When the water is available it must be absorbed rapidly, so that the necessity for the formation of root hairs, first of all, would seem to be a drawback to the plant; hence it seems feasible that water is absorbed more rapidly by osmosis through the thin walled epidermal cells.

About 1 mm. behind the apex, the epidermis and cortex become transformed into sclerenchyma. The root is diarch, the two protoxylem groups occurring at the ends of the xylem plate. The metaxylem elements are few, but very large; they have scalariform thickening laid down on their walls of the same nature as that in the stem and petiole. The phloem immediately surrounds the xylem with a little parenchyma intervening. The sieve tubes are few and do not form a continuous ring around the xylem, the phloem elements being absent in the vicinity of the protoxylem groups (fig. 22). Surrounding this is a pericycle, the cells of which are densely granular, as in other parts of the plant. The endodermis is not always to be seen in the older portions of the root, but where it does occur it is usually dark brown, with thick cell walls. When the root dries, however, the endodermis splits, and the vascular system becomes separated from the ground tissue.

GWYNNE-VAUGHAN (**6**) claims that the ontogeny of the vascular system as a whole is often repeated, although more or less imperfectly, in the development of the lateral shoots. In all the Polypodiaceae which he has examined, the root stele was invariably diarch. *C. tenuifolia* lends additional support to this view.

SORUS

The sori are developed later on the ordinary leaves. They are superficial on the lower surface of the leaf, and bear a close relation to the veins, usually forming above the free end of the small veins. They are protected merely by the inrolled margins of the leaf.

Fig. 23 represents a section through a soral region, and indicates that the receptacle is wide but flat, and from it numerous sporangia arise, all of different ages, younger sporangia interpolated between those already matured. Protecting these is the inrolled margin of the leaf, which may be termed an indusium, since it protects the sori. From its mode of origin and its structure, however, it is nothing more than the result of the outgrowth and thinning out of the margin of the leaf, which has become specialized for protective purposes.

The vascular strands run to the point immediately below the surface of the sorus, and then widen into a considerable mass of tracheids, surrounded by a layer of parenchyma, and limited by an endodermis.

The mode of development of the sporangia conforms with that usually found in the other Mixtae (figs. 24–28). The growth of the sporangium may be traced to a single epidermal cell (fig. 24). This cell protrudes above the surface of the epidermis, and is divided by a transverse wall level with the surface of the receptacle into a basal or stalk cell, and an upper cell which forms the sporangium. This BOWER considers to be the simplest segmentation of all. The basal cell takes no further part in the formation of the sporangium. The outer cell is divided by a strongly inclined wall which meets the basal wall, and this is usually followed by two others, so as to inclose a three-sided cell. From this a number of cells are cut off which form the three rows of cells seen in the stalk. GOEBEL was of the opinion that the stalk was separated from the capsule and formed from the basal cell, but the observations of various investigators on a large number of genera have proved this to be erroneous. The cells of the stalk undergo no further longitudinal divisions, so that it remains permanently composed of three or four rows of cells.

A periclinal wall is formed in the sporangium proper; thus a central cell and the wall cells are differentiated. The wall cells undergo no further periclinal divisions, but repeated radial divisions take place, so that the wall remains permanently one layer thick (fig. 25). Fig. 26 represents a still further advance in development. Here the sporangium has enlarged considerably, the contents of the

central cell (the archesporium) have become densely granular, and it has undergone periclinal division, so that a single-layered tapetum has been cut off. The cells of the tapetum undergo another periclinal division, and a two-layered tapetum is formed. This is constant and never varies. The tapetal cells undergo further divisions by radial walls. Fig. 27 indicates that a two-layered tapetum has been formed, and the archesporium has been divided into two cells by a vertical wall, which nevertheless is inclined to be oblique. In every instance observed this wall was vertical or oblique, but never transverse. A wall is then formed at right angles to this, dividing the archesporium into a quadrant of cells (fig. 28). Further transverse divisions take place in each cell, as a result of which an octant of cells is formed. The cells of the tapetum and archesporium have dense cytoplasm and large nuclei.

The next stage obtained was that in which the tetrads had been formed and the spores had separated. Unfortunately no stages between these could be obtained, but from similarity in other details it would seem probable that reduction division occurs in much the same manner as in the other Polypodiaceae. At maturity the cells of the tapetum disappear, the spores separate and become rounded, while the wall cells become organized into various parts. The number of spores is comparatively small, and this is characteristic of the Mixtae. The spore possesses several walls, the outer or perinium of which is usually brown and folded. The spore contents consist of one large nucelus and densely granular cytoplasm (fig. 29).

As in the other Mixtae, the annulus is vertical and interrupted by the stalk. Its cells have thickened walls, but the thickening does not occur on the outer part of the wall, which remains thin. The cells of the annulus which are situated near the stomium are larger than those occurring farther away. The other cells of the sporangial wall have thin cell walls.

The details of the development of the sporangium coincide very closely with those described for other members of the Mixtae. BOWER (1) considers that "reduction of the indusium has come along more than one line of descent. One such probable line might be traced from the Cyatheaceae with a two-sided indusium to *Hemitelia*, where it is one-sided. This has been responsible for the

exiguous, fimbriated indusium of *Woodsia* and *Hypoderris*. From this it is but a slight step to forms of the comprehensive genus *Polypodium*, where, with similarity of superficial sorus, the indusium is absent." The absence of an indusium in *Cheilanthes* has probably come along some such line of descent.

Conclusion

A study of the anatomy of *Cheilanthes tenuifolia* shows it to be a very interesting type, since in every part it reveals transitional characters. MARSH has shown that in *Cheilanthes* there exist types which are solenostelic, and others which are intermediate between solenostely and dictyostely. *C. tenuifolia* is a true dictyostelic type. Thus in the one genus the gradual transition from solenostely to dictyostely may easily be traced. Although dictyostely does occur, it is a very simple form of this type of anatomy, not comparable in complexity with the ultimate state of dictyostely which has been reached in the vast majority of the Mixtae. These considerations would place the genus among the early types.

The leaf trace and petiolar anatomy also tend to support this view. The leaf trace is single, and of a type characteristic of the solenostele; hence, although dictyostely does occur, it has just evolved, and is little removed from solenostelic types. In *Adiantum* the leaf trace is double, so that in this respect it is more advanced than *Cheilanthes*. The anatomy of the petiole is also simple. The vascular bundle is single, showing tendencies toward doubling, but nevertheless its structure is that characteristic of solenostelic types. GWYNNE-VAUGHAN claims that in stems with advanced dictyostelic structure the petiolar bundle is broken up into two or more portions, although where the structure is little removed from solenostely a single strand is often found. This is precisely what occurs in *C. tenuifolia*.

The leaf does not show reticulate venation, which is held to be a phyletic advance in forms in which it occurs.

ENGLER and PRANTL (4) classify the Pterideae into four suborders (Gymnogramniaeae, Cheilanthineae, Adiantineae, and Pteridineae). This position seems to be quite in accordance with the evidence of the present investigation. The anatomy proves it

to be of a simpler type than that which occurs in *Adiantum*, while *Pteris* has the most complicated leaf trace and dictyostely of all. There is a close relation between apical segmentation and development of the sporangium in all these types.

Cheilanthes is transitional between solenostelic and dictyostelic types of the Mixtae, between the simpler and the more complex. *C. Fendleri* is a true solenostele; the leaf gaps are long, but fail to overlap. *C. gracillima* is a true solenostele for the greater part of its course, but sometimes extra leaves are inserted. In *C. Lendgeri*, a type described by GWYNNE-VAUGHAN, each leaf gap is closed before the next leaf trace arises, so far as the phloem, endodermis, and pericycle are concerned, but the leaf gap in the xylem remains open; hence they overlap. In *C. lanuginosa* the stem is a dictyostele for the greater part, although sometimes the section is a perfect ring. *C. persica* and *C. tenuifolia* are true dictyostelic types. Right through this series, however, the leaf trace does not advance, but remains in the condition typical for a solenostele.

Every part of the plant shows adaptations to the xerophilous habitat. The subcoriaceous texture, the inrolled margins of the leaves (which, as well as serving as a protection for the sori, serve to control excessive transpiration from the stomata), stomata confined to the lower surface, all indicate well marked adaptations. The phloem is very much reduced in every part of the plant, hardly ever consisting of more than a single layer of sieve tubes. The pericycle is abnormally developed, and its cells are stored with reserve food, so that in this respect it serves as a storage parenchyma. The xylem parenchyma has also reserve food supplies. The cortex of stem, petiole, and root is highly sclerized.

From every standpoint *C. tenuifolia* proves to be a very interesting type, on account of its well marked xerophily, exceptional among ferns, and its characters intermediate between solenostely and dictyostely.

Summary

1. The apex of the rhizome is occupied by a tetrahedral apical cell.

2. The stelar anatomy of the stem is dictyostelic. The xylem is broken by wood parenchyma. There is no protoxylem present,

the first elements to be lignified being large, scalariform tracheids.

3. The amount of phloem present in the stem is small, consisting of a single row of sieve tubes surrounding the xylem, the amount of external being equal to the amount of internal phloem.

4. The pericycle is well developed, and has reserve food within it.

5. The apex of the leaf is occupied by a wedge-shaped apical cell.

6. The vascular strand of the petiole is single and concentric.

7. The petiole may be derived from types with triarch, mesarch protoxylem, described by SINNOTT. The medium protoxylem group moves its position, becomes endarch, and finally completely detached.

8. The leaf has the stomata localized to the lower surface, protected by the inrolled margins of the leaf alone. The cuticle and palisade tissue are well developed, while the lacunar tissue is reduced.

9. The root is endogenous in origin, and is formed by segments cut off from the three-sided apical cell.

10. The root is diarch, new branches arising in two lines corresponding to the ends of the xylem plates. The phloem is poorly developed, while the pericycle is well developed.

11. The sori are marginal, and are protected by the inrolled margins of the leaf; no true indusium is present.

12. The development of the sporangia corresponds mainly with that found in the other Mixtae.

13. The mature sporangium has a vertical annulus interrupted by the stalk.

The writer wishes to express thanks to Professor A. A. LAWSON, in whose laboratory the investigation was carried out, for kindly criticism throughout the work; and also to Dr. MCLUCKIE and Mr. BROUGH for helpful suggestions.

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EXPLANATION OF PLATES VIII–X

FIG. 1.—Longitudinal section of apex of stem, showing cleavage of apical cell which will apparently give rise to dichotomous branching; ×202.

FIG. 2.—Longitudinal section of apex of stem, showing tetrahedral apical cell and segmentation of same; scales present protecting apex; ×202.

FIG. 3.—Same as preceding; ×460.

FIG. 4.—Transverse section of stem made near apex; stelar anatomy dictyostelic; root and leaf traces being given off; cortex and pith not yet sclerized; ×25.

FIG. 5.—Transverse section of meristele of dictyostele; xylem seen in center surrounded by phloem and pericycle; ×45.

FIG. 6.—Transverse section of portion of meristele, showing nature of xylem, internal and external phloem, and a few of cells of pericycle; ×202.

FIG. 7.—Longitudinal section of rhizome, showing xylem, phloem, pericycle, and endodermis; ×112.

FIG. 8.—Longitudinal section of apex of leaf, showing wedge-shaped apical cell and segmentation from this; ×202.

FIG. 9.—Transverse section of petiole, showing single vascular strand; ×45.

FIG. 10.—Transverse section of portion of vascular strand of petiole, showing median protoxylem group dividing unlignified metaxylem elements into two masses; other protoxylem groups endarch; ×112.

FIG. 11.—Transverse section of vascular strand of petiole made further up on older stem; metaxylem here is single continuous mass; medium protoxylem group has become detached; ×112.

FIG. 12.—Transverse section of portion of petiolar strand, showing nature of xylem, external and internal phloem, and a few of pericycle cells; ×202.

FIG. 13.—Longitudinal section of vascular strand of petiole, showing nature of metaxylem and phloem; ×112.

FIG. 14.—Transverse section of pinnule, showing upper and lower epidermis, stomata confined to lower surface, palisade parenchyma, and lacunar tissue and vascular bundle; ×112.

FIG. 15.—Surface section of lower epidermis, showing nature of stomata and convoluted outline of epidermal cells; ×112.

FIG. 16.—Transverse section of vascular bundle of pinnule; endodermis well marked and surrounds bundle; xylem surrounded by phloem and pericycle; ×202.

FIG. 17.—Diagrammatic representation of longitudinal section of apex of stem, showing endogenous origin of root, also apex of stem and petiole; ×25.

FIG. 18.—Portion of longitudinal section of apex of the stem, showing endogenous origin of root, also apex of root and petiole; ×112.

FIG. 19.—Longitudinal section of root apex just emerging from stem, and still surrounded by scales of latter; ×202.

FIG. 20.—Longitudinal section of root apex, showing apical cell of root, root cap, and root tissue; ×112.

FIG. 21.—Transverse section of young root, showing two protoxylem groups already lignified; remainder of tissue thin walled; endodermis well marked; ×45.

FIG. 22.—Transverse section of vascular bundle of root, showing diarch nature, also nature of xylem, phloem, and pericycle; ×112.

FIG. 23.—Section through soral region, showing longitudinal section of vascular commissure, inrolled margin of leaf, and sporangia; ×112.

FIG. 24.—Longitudinal section of young sporangium, showing development from single epidermal cell, which possesses very large nucleus; ×202.

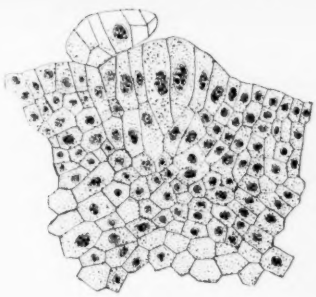
FIG. 25.—Longitudinal section of sporangium in which wall cells and stalk have been cut off, and archesporium consists of single cell; ×202.

FIG. 26.—Further development of preceding; single-layered tapetum cut off; ×202.

FIG. 27.—Transverse section of pinnule, showing transverse section of vascular commissure to sorus; further advance in development of sporangium also presented; tapetum two-layered; archesporial cell divided by slightly inclined vertical wall; ×112.

FIG. 28.—Longitudinal section of sporangium still further developed; each of two cells shown in previous diagram has undergone division by transverse wall, so that quadrant of cells is formed; ×202.

FIG. 29.—Section of spore, showing folded outer coat, large nucleus, and granular cytoplasm; ×202.



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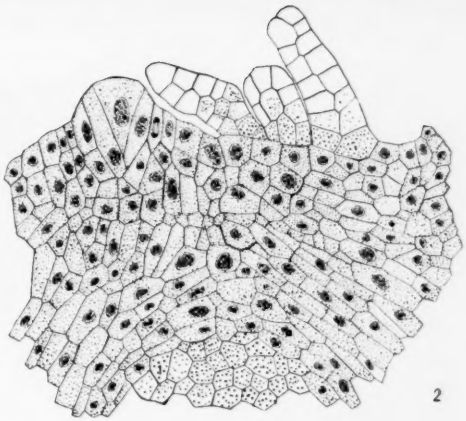

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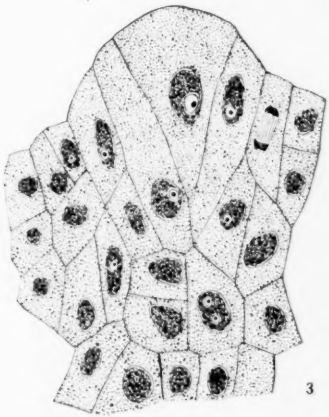

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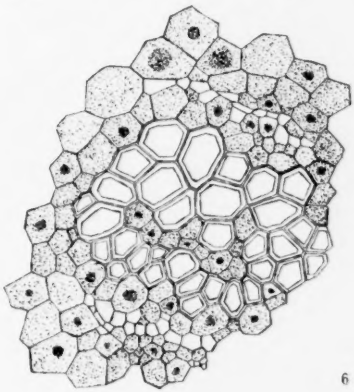

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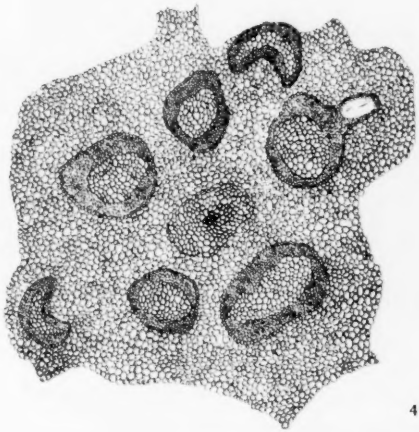

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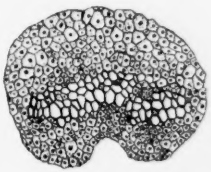

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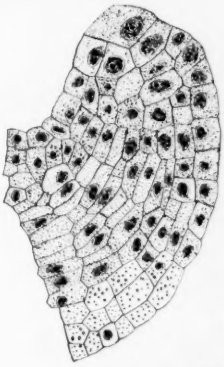

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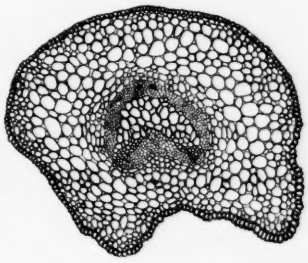

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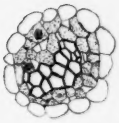

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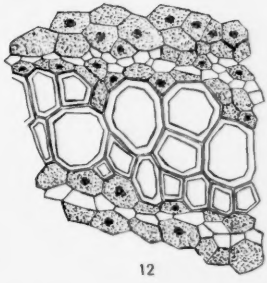

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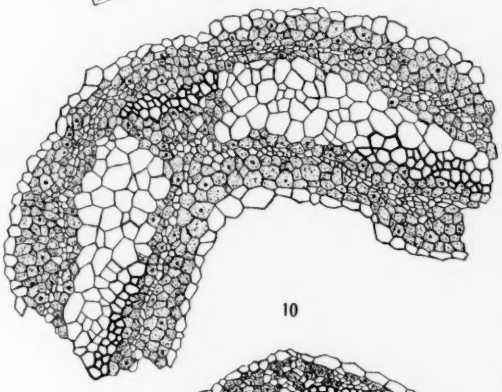

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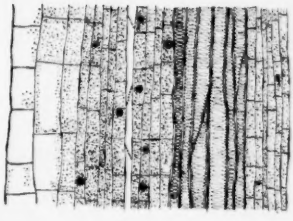

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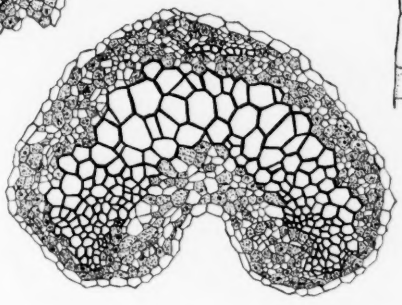

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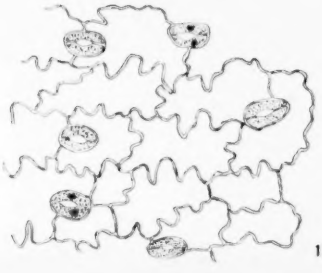

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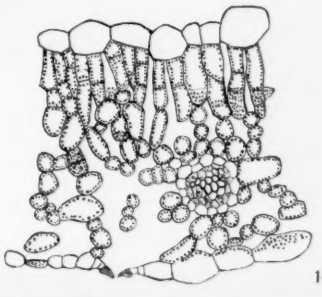

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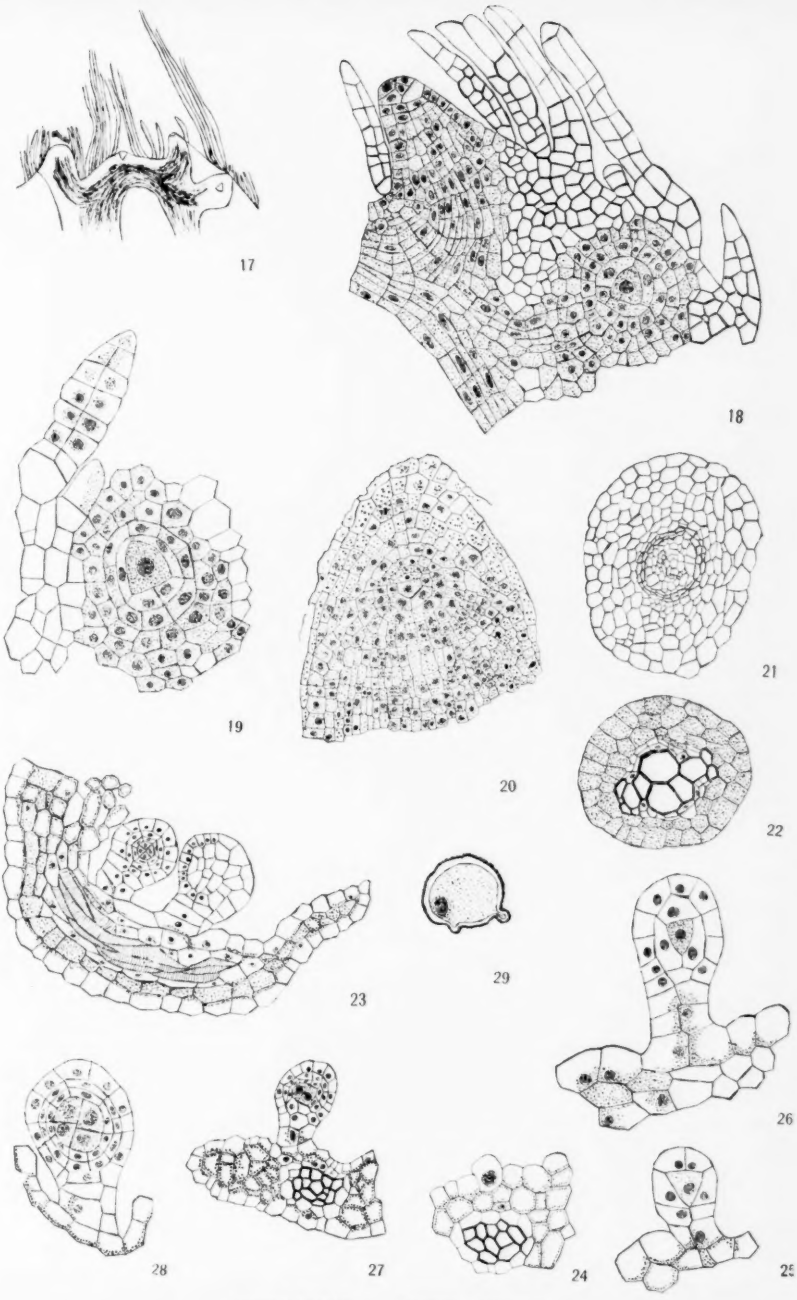

WILLIAMS on CHEILANTHES

EFFECT OF SIZE OF SEED ON PLANT PRODUCTION WITH SPECIAL REFERENCE TO RADISH

N. A. ROTUNNO

(WITH FOUR FIGURES)

Introduction

The effect of the size of seed on plant production, that is, crop yield, is an old problem. The literature reveals many references to the subject, together with varying results and differing opinions. In many cases the conclusions drawn are based on insufficient data when compared with the standards set by present day investigation. The methods employed by the numerous investigators have differed widely, consequently many of the results are not comparable.

The major part of the work has been done with cereals, especially wheat and oats. In the selection of experimental material for this work the writer gave consideration to those plants of which several crops could be grown in the greenhouse in one season. *Raphanus sativus* was selected as fulfilling this requirement. Very little work has been done with the radish, and the investigators have quite uniformly concluded that the large seed is superior to the small seed. The number of seeds used in most cases was comparatively small, and the weights of the resultant crops were usually taken en masse.

After preliminary experiments, it seemed desirable to obtain data with reference to the following questions: (1) Are weights of various sizes of seed comparable in different varieties? (2) Is there a difference in the percentage of germination between the large, medium, and small seed? (3) Which size of seed produces the heaviest roots? (4) Which size of seed produces the most marketable roots? (5) Are the results obtained with one variety true of another? (6) Is it profitable commercially to screen seed, and which size or sizes should be rejected if any? (7) Is there a correlation between the weight of the plant and the size of the root?

Historical

Among the earliest investigators who believed that the large seed was superior to the small was GALLOWAY (**7**), who worked with radish, using a total of 400 to 500 seeds. HICKS and DABNEY (**12**) reached the same conclusion on experiments with vetch, radish, rye, kaffir corn, and oats, using from 50 to 100 seeds of the large, medium, and small sizes for each crop. Since the number of seeds they used was small, and the experiments continued only for 24 days with radishes, 15 for vetch, 39, 23, and 23 for kaffir corn, rye, and oats respectively, and since in that time none of the crops tested had matured, the conclusions drawn are not entirely justified.

SHAMEL (**18**), working with tobacco, but making no statement as to the number of seeds used, finds that "careful comparative tests of light and heavy seed have proved that the best developed and the more vigorous plants are always produced by the large, heavy seed, while the small, light seed produces small, irregular, and undesirable plants."

EISENMENGER (**5**), experimenting with seeds of spruce, Scotch and Austrian pine, found that the large seeds germinated in shorter time and reached the period of germination earlier than small seeds, but that dark seeds gave a higher percentage of germination than light colored ones. SNYDER (**19**), in discussing light and heavy seeds of barley, wheat, and oats with particular reference to their composition, deduces from his observations that large seed is superior to small, or "that the farmer is repaid for using heavy weight seed." However, no actual data were presented as to tests on which he based his conclusion.

BURNETT (**2**), after extensive experiments on oats, using a large number of seeds of twenty varieties, states that "the figures tend to show that the heavy and light seeds were about equal, pound for pound, but not seed for seed, nor measure for measure."

HAYS (**10**), commenting on the results obtained from one test with oats, found that the heavy seeds produced more than the light. CUMMINGS (**3**), in discussing this subject after working with a large number of truck crops, made this general statement: "a distinct advantage follows the use of large seed or heavy seed." In the

same paper, with especial reference to the radish, with which he carried on sixteen different trials extending over a period of two years on two varieties, he states that large seed is superior to small. Not all the data of these trials were presented in this paper. In those recorded, CUMMINGS used in many cases only a small number of seeds, but in one case several thousands were used. The weighings were evidently taken en masse, and the comparisons made only between large and small seeds, although medium sized seeds were used in a few trials.

KIESSELBACH and HELM (**13**), working on a large scale with wheat for a period of ten years, conclude that "in general, results seem to indicate that when space is planted to permit maximum development a higher individual plant yield is obtained from large than from small seed."

Another group of investigators maintain that while the size of seed is an important factor, the origin and strain of the seed are of more importance than the size. Thus FINDLAY (**6**) found in his work with oats, barley, turnip, and red clover that in a single sample of seed the large produced more than the small, but in different samples of the same seed the strain and origin are of more importance than the size.

A third group of investigators believe that medium sized seed is superior to large and small. Among these are HICKMAN (**11**), who worked with three varieties of wheat, and used "selected seed consisting of the largest grains, the second grade the best of the wheat that passed through in screening out the first grade, and the unscreened seed, the entire lot as it came from the thresher," found that the second grade gave the highest yield in bushels per acre and in weight per bushel over large or unscreened seed in experiments carried on for a period of nine years. HARRIS (**8**), who experimented with *Phaseolus vulgaris*, concludes as follows: "In short, both large and small seed are less capable of developing into fertile plants than those which do not deviate so widely above or below the type." In a later paper (**9**) he states that "as the weight of the seed increases the time for germination becomes longer."

A fourth group of investigators are undecided in their opinions as to which size is best. Thus MONTGOMERY (**15**) finds "that after

eight years of continuous selection by the fanning mill, it was not possible by careful examination to note the difference in either the quality or quantity of the crop produced from the light and heavy seed." In a later paper (**16**), basing his conclusions on the results obtained on yield from one hundred plants of "large or plump" and "small or undeveloped" seed of both wheat and oats, he states that "small seed were at a marked disadvantage."

WILLIAMS (**21**), working with winter wheat, using twelve 6.5 inch pots planted with hand-sorted seed of large and small grains, and planting eight grains in each pot, found that the small seed produced more grain than the large seed. In drawing his conclusions, he says "the selection of grains, whether by means of the fanning mill or by hand, does not seem to promise anything in the way of permanent improvement in wheat in normal seasons." Later, in a paper on oats (**20**) in which the work was carried on for seven years, and evidently working with a large number of seeds, he finds that the large seed produced four bushels more per acre than small seed.

WILLIAMS and WELTON (**22**), using first, second, and third grade seeds of wheat and planting them at uniform rates on an extensive scale, conclude that when heavy and light seeds are separated by the fanning mill method there is not much hope of ever or even temporarily improving the yield of wheat; but with their work with oats (**23**), which was also done on a very large scale, they find large seed superior to small.

KIESSELBACH and RATCLIFF (**14**), experimenting with American Banner oats, using 1/30 to 1/15 acre plots, find that "as an eight year average the light seed has yielded 1.44 bushels more than the heavy." In the same paper, however, using the Kherson variety, they report that the large gave better results than the small seed. NIELSEN (**17**), after working with rye for ten years and using from 181.6 to 207.1 pounds of seed per acre, states that "in spite of the great difference in size of the seed the increase in the yield from the large seed was only slight."

It is evident that there are decided differences of opinion as to which size of seed produces the best results. It seemed logical,

therefore, to investigate further, using a large number of seeds, and growing the crops at different seasons of the year.

Materials and methods

The work here described was done in greenhouse benches 4 feet wide and 6 inches deep (fig. 1). Temperature and moisture conditions were kept as near the ideal as possible, although the former fluctuated at times. Before each sowing, the soil, a well prepared greenhouse soil, was spaded, raked, and prepared for planting. Rows were marked off 6 inches apart and half an inch deep.

In the first three series of plantings the seeds were divided into two groups only, large and small. The large or heavy seeds may

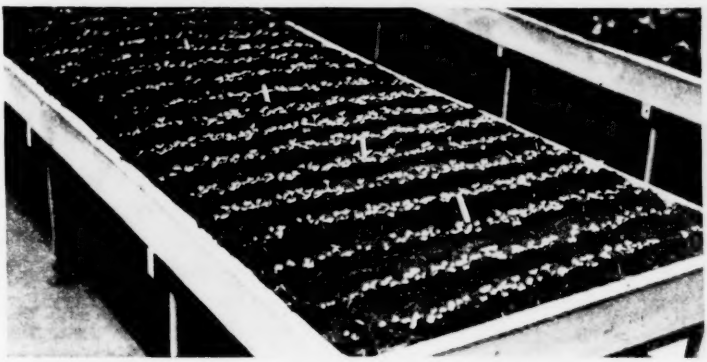

FIG. 1.—Greenhouse bench, showing how crops were grown; Scarlet Globe seedlings from small, medium, and large seeds.

be described as those which did not pass through a 12 mesh sieve. The small or light seeds are those which did pass through the sieve. Later the seeds were divided into three sizes, large, medium, and small. In these cases the large or heavy seeds were those which did not pass through a "chess" sieve, the openings of which were 2.1×12.7 mm.; the medium or middle weight seeds were those which did pass through this, but which would not pass through a 12 mesh sieve; and the small seeds were those which passed through the 12 mesh sieve and were the lightest of all.

This method of sorting gives a very distinct demarcation between the three sizes. Different methods have been used by other experimenters, the commonest being the fanning mill method, in which the light seeds are separated from the heavy seed. Another means of separating is the water method, in which the large seeds sink first. A modification of this is the use of liquids having various specific gravities. The writer believes the screen method to be superior to the others, in that it is more rapid and reliable, gives wide variations in sizes, and is more practical.

The varieties used in this work were the "Button Type" radishes suitable for forcing. The Scarlet Globe was used in the first trials because of its popularity locally for forcing. In later experiments, from one to five plantings were made of each of the following: Vick's Crimson Giant forcing, Burpee's Hailstone, Burpee's Scarlet Button, and Burpee's Rapid Red.

A total of twelve series was made. The seeds were sown about one inch apart, or 50 seeds in each row, which ran the width of the bench. Germination records were kept in all cases by daily notations which began with the appearance of the first seedling above ground.

When the majority of the plants had reached marketable condition, they were pulled, washed, and the surplus water allowed to run off. First the weight of the whole plant was recorded, and then the root was cut from the plant and weighed. These two weights were kept together for future references. The roots designated as salable were those which weighed at least 3 gm., and which had the characteristics of the variety they represented. The weights were taken to 0.01 gm., using a "Cenco" triple beam balance which is sensitive to 0.01 gm.

Records for each series were kept as follows: (1) average weight of 100 seeds of the large, medium, and small of each variety; (2) germination percentage; (3) weight of plant; (4) weight of roots from each plant; (5) average weight of the plants; (6) average weight of the roots; (7) biometric tabulations determining correlation between the weight of the plants and the weight of roots (in six series only); (8) number and percentage of salable plants from each size of seed. Records were kept of the date of planting, date

of harvesting, and the number of seeds of each size planted in each series. The data are given in table I.

TABLE I

Series	Variety	Date of planting	No. of seeds planted			Date of harvest	No. of growing days
			Large	Medium	Small		
I*...	Scarlet Globe	2/ 2/22	100	.....	100	3/13/22	40
II....	Scarlet Globe	3/16/22	300	.....	200	4/21/22	36
III....	Crimson Giant forcing	3/16/22	200	.....	200	4/24/22	39
IV....	Scarlet Globe	10/15/22	150	150	150	12/19/22	65
V....	Scarlet Globe	10/15/22	200	200	150	11/ 7/22	23
VI....	Crimson Giant forcing	10/15/22	150	150	150	11/ 7/22	23
VII....	Hailstone	1/26/23	150	150	150	3/16/23	49
VIII....	Crimson Giant forcing	3/ 1/23	200	200	200	4/ 9/23	39
IX....	Scarlet Globe	3/ 1/23	150	150	150	4/ 5/23	35
X....	Rapid Red	3/16/23	200	200	200	4/22/23	37
XI....	Hailstone	3/17/23	100	100	100	4/22/23	36
XII....	Scarlet Button	3/17/23	100	100	100	4/23/23	37

* In series I no count was kept as to the number of seeds sown. The plants were thinned to 100 of each from the large and small seeds and in each case the best plants of the lot were left. In all the other series, however, the exact count of the seeds was recorded in each case.

Results

Relation of size to weight

As previously described, the seeds were separated into the groups large, medium, and small, and ten samples of 100 seeds of each size were counted, weighed, and the average weight of 100 seeds computed. This was done with all the varieties discussed in this

TABLE II

Average weight in gm. of 100 seeds computed from 10 samples

Variety	Large	Medium	Small
Scarlet Globe	1.06	0.74	0.49
Crimson Giant forcing	1.09	0.79	0.59
Hailstone	0.90	0.74	0.55
Rapid Red	0.93	0.61	0.42
Scarlet Button	0.95	0.69	0.51

paper. The results obtained are given in table II. A very distinct direct relation of size to weight seems to exist in all the varieties. The seed designated as medium is about two-thirds the weight of

the large, and the small seed about two-thirds the weight of the medium. The relative sizes of the seeds are shown in fig. 2.

GERMINATION

Much has been written about the difference in germination between the large and small seed, and in almost all cases it is claimed

FIG. 2.—Seed of Scarlet Globe variety, showing relative sizes of large, medium, and small seeds; slightly reduced.

that the large seed has a decidedly higher percentage of germination than the small. Since some differences of opinion were noted in reviewing the literature, germination records were kept to substantiate or disprove the general opinion held on this point. Table III presents the data obtained in these tests.

From Table III it is evident that there is a decided variation in the percentage of germination between the various sizes of seeds. Considering the series singly, there are cases in which the large have an advantage over the medium and small, and in other cases the medium or small show an advantage over the others. As will be noted, the large seed of the Scarlet Globe variety shows a slightly higher percentage of germination over the medium and small seed. In the Rapid Red variety the small seed has a marked advantage over the large and medium, whereas in the Crimson Giant variety

TABLE III

Series	Variety	No. of seeds used			No. germinated			Percentage germination		
		Large	Medium	Small	Large	Medium	Small	Large	Medium	Small
II	Scarlet Globe	300	.....	200	246	.....	143	82.0	.....	71.5
IV	Scarlet Globe	150	150	150	127	111	110	84.6	74.0	74.0
V	Scarlet Globe	200	200	150	133	146	106	66.5	73.0	70.0
IX	Scarlet Globe	150	150	150	139	132	114	90.2	89.0	76.0
III	Crimson Giant	200	.....	200	143	.....	153	71.5	.....	76.5
VI	Crimson Giant	150	150	150	121	103	123	80.0	70.0	80.2
VIII	Crimson Giant	200	200	200	129	117	124	64.5	58.5	62.0
VII	Hailstone	150	150	150	120	131	86	80.0	87.3	57.3
XI	Hailstone	100	100	100	70	72	83	70.0	72.0	83.0
X	Rapid Red	200	200	200	72	73	104	36.0	36.5	52.0
XII	Scarlet Button	100	100	100	28	27	24	28.0	27.0	24.0
Total		1900	1400	1750	.....	.....	.....	.....	.....	.....
Average		.....	.....	.....	.....	.....	.....	68.48	70.03	66.50

the small seed shows a slight increase over the large and medium seed when averaged. In the Hailstone variety, when the percentages are averaged, the medium shows a decidedly higher percentage of germination than either the large or small, while in the Scarlet Button variety the large and medium size germinate about the same, and both show slightly better germination than the small seed. The average for each of these sizes for all the varieties, taken as a whole, indicates that the difference between them is slight and within limits of experimental error.

Correlation between weight of plants and roots

So far as could be ascertained, no one has reported as to whether a correlation exists between the weight of the plant and the weight

TABLE IV

COMPARISON OF RESULTS OF 12 SERIES OF PLANTINGS

Series	No. harvested	Total weight in gm.	Average weight of plants	Weight roots	Average weight	No. salable	Percentage salable
I:* *Scarlet Globe*							
Large	100	829.90	8.29	312.50	3.1200	41	41.0
Small	100	906.20	9.06	396.40	3.9600	55	55.0
II: *Scarlet Globe*							
Large	246	1639.25	6.60	506.10	2.050	64	26.0
Small	143	821.00	5.70	202.80	1.600	24	16.5
III: *Crimson Giant forcing*							
Large	143	1730.11	12.10	525.90	3.600	55	38.4
Small	153	1880.67	12.30	572.70	3.700	68	44.4
IV: *Scarlet Globe*							
Large	127	720.90	5.60	206.20	1.710	23	18.0
Medium	111	768.78	6.90	275.12	2.480	46	41.0
Small	110	566.50	5.10	151.90	1.380	16	14.0
V: *Scarlet Globe (3 weeks old)*							
Large	133	122.85	0.91	13.83	0.100	.....	.....
Medium	146	183.96	1.26	22.75	0.160	.....	.....
Small	106	108.11	1.02	13.27	0.125	.....	.....
VI: *Crimson Giant forcing (3 weeks old)*							
Large	121	195.20	1.61	12.58	0.104	.....	.....
Medium	103	174.10	1.69	9.40	0.090	.....	.....
Small	123	185.00	1.50	13.83	0.112	.....	.....
VII: *Hailstone*							
Large	120	561.00	4.70	217.10	1.800	26	21 6
Medium	131	738.50	5.60	280.48	2.140	32	24.4
Small	86	263.08	3.05	101.16	1.170	9	10.4
VIII: *Crimson Giant forcing*							
Large	129	1442.74	11.17	386.53	3.040	45	34.9
Medium	117	1359.25	11.60	370.75	3.190	44	37.6
Small	124	1376.15	11.10	401.77	3.280	49	39.5
IX: *Scarlet Globe*							
Large	139†	1221.90	8.79	453.85	3.260	69	50.0
Medium	132	1072.28	8.12	435.47	3.300	75	56.7
Small	114	576.15	5.10	235.09	2.060	35	30.7
X: *Rapid Red*							
Large	72†	496.91	6.90	315.53	4.370	47	65.2
Medium	73	527.08	7.22	369.44	5.060	62	85.0
Small	104	403.39	3.88	240.65	2.310	38	36.5

* In series I only the best plants were selected and allowed to mature.

† Two plants in each of these series were off type, therefore are not included.

TABLE IV—*Continued*

Series	No. harvested	Total weight in gm.	Average weight of plants	Weight roots	Average weight	No. salable	Percentage salable
XI: *Hailstone*							
Large	70	327.35	4.67	165.81	2.360	25	35.7
Medium	72	454.89	6.31	215.48	2.990	39	54.1
Small	83	536.60	6.46	260.98	3.140	41	49.4
XII: *Scarlet Button*							
Large	28	176.27	6.29	91.29	3.26	11	39.3
Medium	27	148.30	5.49	76.65	2.83	12	41.5
Small	24	139.30	5.8	72.61	3.02	11	45.8

of the root in the radish. Since the individual weight of the plants and their roots had been recorded, it was thought advisable to determine whether or not a correlation existed between these two weights. Biometric tables were arranged using one gram classes. Thus all plants or roots weighing between one and two grams were placed in the 1.5 class, those weighing from four to five grams were placed in the 4.5 class, etc. Table V represents the results of the correlations from series 2, 3, 4, 5, and 6 for the large, medium, and small seeds.

TABLE V

Variety	Series	Correlation coefficients from		
		Large	Medium	Small
Scarlet Globe	2	+0.91±0.01	.....	+0.83±0.016
Crimson Giant forcing	3	+0.89±0.01	.....	+0.88±0.012
Scarlet Globe	4	+0.82±0.019	+0.74±0.025	+0.80±0.023
Scarlet Globe	5	+0.46±0.05	+0.80±0.02	+0.67±0.03
Crimson Giant forcing	6	+0.59±0.04	+0.58±0.04	+0.67±0.03

Since it is generally agreed among biometricians (1) that, to be significant, the ratio of correlation should be at least six times the probable error, it appears that a definite correlation exists.

Discussion

In the majority of papers cited there are usually no data to indicate the difference in weight between the large, medium, and small seeds. It is possible that in tests where wheat is used the

small seed is the lightest in weight, but with oats the relation between size and weight may not hold, as it is possible that a large oat seed may not weigh as much as one which is smaller. Table II, however, indicates that with radish seeds a distinct relation exists between size and weight when screened, according to the methods previously described. The differences are so marked and distinct that they can justly be called "large, medium, and small" seeds, or "heavy, medium, and light" seeds. In other words, "large, medium, and small" as to size correspond with "heavy, medium, and light" as to weight.

With reference to the differences in percentage of germination between large, medium, and small seeds of radish, GALLOWAY (7) and CUMMINGS (3), basing their conclusions on results obtained from two varieties, say that large seeds give a higher percentage of germination than small seeds. Variety differences, however, have appeared in the present work. From the data presented in table III it appears that the varieties used in these experiments group themselves into three classes, as follows:

First, in the Scarlet Globe variety the large seeds give a higher percentage of germination than the small when only large and small seeds are used. When the seeds are divided into three groups, however, large, medium, and small, the large gives only a slightly higher percentage of germination than the medium, and both have an advantage over the small seeds. Likewise, with the Scarlet Button variety the large have a very slight advantage over the medium, and again both are superior to the small seeds.

In the second group are those varieties in which the medium seeds are superior to the large or small. Thus in the Hailstone variety, as an average for two trials, series VII and XI representing altogether 750 seeds, the medium seeds give the highest average percentage of germination.

In the third group, which includes the Crimson Giant forcing and the Rapid Red varieties, the small seeds quite uniformly give the best germination when the seeds are divided into large, medium, and small sizes. With the Crimson Giant forcing variety the small seeds germinate best, even though only large and small seeds are used (series III).

It is evident, therefore, that the percentage of germination of the large, medium, and small seeds differs with the variety. When the percentages are averaged for all the series, however, they are quite uniform, and no one size has a great advantage over the others. These results when taken as a whole do not agree with those obtained by previous investigators. Since, however, the number of seeds used is sufficiently large, and since more varieties are included in the present work than in any previous work on radishes, the results may be regarded as significant.

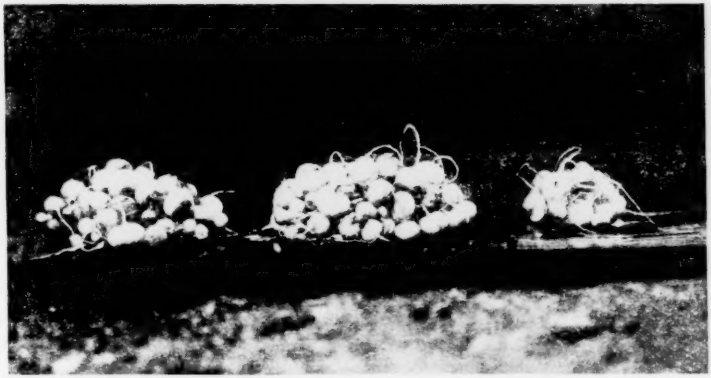

FIG. 3.—Roots from 150 seeds each of large, medium, and small seeds of Hailstone (reading from left to right).

In growing radishes commercially, the important part of the plant is the root. The smaller the tops and the larger the roots, within certain limits depending on the market and the variety, the better the sales. Of course, the larger the photosynthetic tissue the more rapid the growth, other things being equal. For this reason it was interesting to note which size produced the best product. A summary of the results obtained with the twelve plantings is shown in table IV. It will be seen that the results from the different sizes again group themselves according to variety.

In the one trial of the Scarlet Button, the large seeds produced a slightly heavier root than the medium or small seeds. With the Scarlet Globe variety (series II), when large and small seeds only

are used, the large produce a heavier root than the small, but when large, medium, and small seeds are used (series IV and IX), the medium seeds produce a decidedly heavier average root (2.89 gm.) than either the large (2.48 gm.) or the small (1.72 gm.).

In the two series of the Hailstone variety, one of the trials shows that there is a strong tendency for the medium seeds to produce the heaviest roots, while in a trial made later in the spring the small seeds produced the heaviest roots with the medium a close second. The average for the two series shows that the medium seeds produced the heaviest roots. In the several trials with the Crimson Giant forcing

FIG. 4.—Roots from 200 seeds each of large, medium, and small seeds of Crimson Giant forcing variety (reading from left to right)

variety, the plants from the small seeds in every case produced heavier average roots than the large or medium seeds. The Rapid Red variety seems to exhibit the same tendency as the Crimson Giant forcing.

It is evident, therefore, that there is no uniformity of results from seeds of the same size in different varieties, and therefore they do not conform to the results of previous investigators.

With regard to the percentage of salable roots produced from large, medium, and small seeds, there seems to be as much variation as with the other characters. The trials in which the Scarlet Globe variety was used show that when only large and small seeds are used, the large produce a higher percentage of salable roots

than the small (series II); but when the three sizes, large, medium, and small are used, the medium produce the highest percentage of salable roots. The same phenomena hold true with the Hailstone (fig. 3) and Rapid Red varieties. In the Crimson Giant forcing variety (fig. 4) the small seeds in every case produce the highest percentage of salable roots. The same is true of the one trial of the Scarlet Button variety.

Summary

This paper is to be regarded as a preliminary report on seed studies on the radish. Observations for a number of seasons and on a larger number of seeds should be made to settle definitely some of the phases of the subject touched on in this paper. From the data presented, however, the following conclusions seem justified:

1. The relative weights of the same sizes of seeds in different varieties differ widely, but the relative weights of the large, medium, and small seeds are approximately the same in all varieties.

2. The percentage of germination varies considerably with the large, medium, and small seeds. In some varieties the large seeds germinate best, in others the medium, and still others the small. When the average percentage of germination for the various sizes of the several varieties is computed, the difference between any of the sizes is negligible and within the limits of experimental error.

3. No definite statement can be made as to which size produces the heaviest root. In the majority of trials with the large, medium, and small seeds of the Scarlet Globe, Hailstone, and Rapid Red varieties the medium seeds gave the best results; with the Crimson Giant forcing variety the small seeds always produced the heaviest roots; and in the one trial of the Scarlet Button variety the large seeds produced the heaviest roots. When taken as a whole and averaged, the medium seeds produce the heaviest roots.

4. With reference to the percentage of salable roots, the medium seeds in every variety, except the Crimson Giant forcing, produce the highest percentage of salable roots.

5. The question as to why in one variety the small seeds should give the largest roots, and other varieties the large, and in still others the medium remains unanswered. Theories might be formu-

ated, but the first requisite should be additional dependable data.

6. Apparently there is a definite correlation between the weight of the plants and the weight of the roots.

7. From the data herein recorded it seems established that the results obtained with one variety are not necessarily comparable with another variety, as stated by GALLOWAY (7) and CUMMINGS (3).

The writer takes pleasure in extending thanks to Professors HENRY F. A. MEIER and C. C. CARPENTER for kindly suggestions and criticisms freely given throughout the progress of the work.

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APPLE TUFTS

C. C. CARPENTER

(WITH SIX FIGURES)

Introduction

Apple tufts are the white growths that appear on the carpel walls and seeds of some varieties of apples. They are found along fissures in the carpel walls, which generally run at right angles to the longitudinal axis of the fruit, and upon the seed as a white cotton-like growth. Under the microscope the strands appear separate, almost hyaline, and upon almost every cell wartlike protuberances are found in numbers (figs. 1, 2).

FIG. 1

FIG. 2

FIGS. 1, 2.—Fig. 1, typical tuft strand showing protuberances on cell walls and characteristic manner of branching; fig. 2, strand of tuft tissue showing manner of development; ×200.

In the literature consulted, SORAUER (8) has given more attention to apple tufts, or "Wollstreifen" as he calls them, than any other investigator. He considers the tufts a non-parasitic apple disease, and states that it is more common and is produced in greater abundance in wet seasons, and is of the opinion that it enters the fruit through the calyx cavity at some time early in its development, and that it is a factor in causing apples to decay. He believes that

when it occurs the fruit must be used soon or the loss from decay will be great. His drawings show the characteristic protuberances on the two or three terminal cells of a tuft strand, and the presence of leucoplasts such as may be found in fresh material. SORAUER also shows detached areas of the thick celled carpel wall as is shown in figs. 3 and 4 of this paper. He describes in some detail the results secured by using different stains on tuft tissue.

BEACH (1) uses tufts as one of the characters in his apple descriptions, and states that they are found in many varieties. He offers no explanation as to their structure, composition, or origin. HEDRICK (5) mentions tufts repeatedly as a variety characteristic. They are shown in photographs in technical bulletins (6), but no mention is made of them in the text of the bulletins consulted. They are also shown in certain varieties in hand paintings by EMMONS (3).

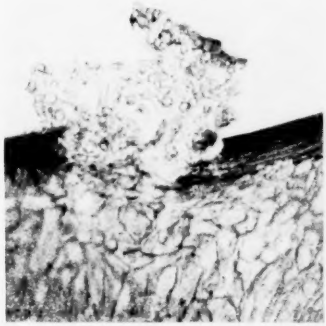

FIG. 3.—Tallman: cross-section showing detached portion of carpel wall dislocated by tuft tissue; ×100.

In some varieties, such as Tallman, R. I. Greening, and Baldwin, tufts are nearly always present. In 319 specimens of Tallman, the writer found only two fruits, or less than 1 per cent, that did not show tufts. In R. I. Greening only one fruit in 146 did not show tufts. Jonathan, both New York and Northwestern grown, failed to develop tufts, although some apples were found that showed the characteristic fissure through the carpel wall along which tufts develop. Northern Spy is variable in regard to this character. Samples of this variety were taken from three rather widely separated locations, Syracuse, South Onondaga, and Geneva, which showed tufts as follows:

	SYRACUSE	SOUTH ONONDAGA	GENEVA
Number of fruits	639	163	153
Percentage showing tufts	41.6	38.7	44.9

It seems that the presence of tufts in Northern Spy was fairly uniform under different environments and in different localities for

the year 1922. Whether this variety uniformly develops them in these percentages would have to be determined by counts over a period of years. This was a wet season in Central New York. According to SORAUER, one should expect a smaller percentage of specimens to show tufts in a dry year. Northern Spy is a good variety in which to study this phenomenon, although probably others are equally good.

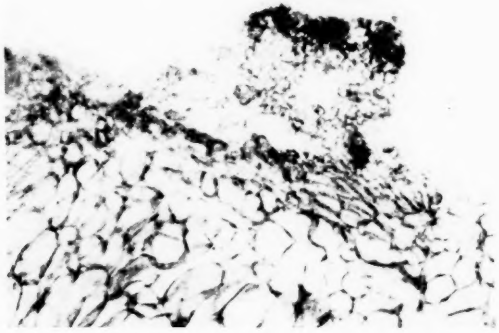

FIG. 4.—Newtown: longitudinal section through carpel fissure showing detached area of carpel wall dislocated by tuft; ×100.

Correlation of tufts with other morphological characters

OPEN CORE.—In talking with orchardists and pomologists, the statement has been made a number of times that tufts are generally found in open core apples. Desiring to determine in regard to this, upon examination it was found that 215 of the varieties of apples in the *Cyclopedia of Hardy Fruits* were so described for core characters that correlation could be determined. In this publication the core was described as open, partly open, open or closed, closed or open, and closed. It was supposed that when varieties were recorded as being found with both open and closed cores, the commonest type was placed first in the description, and was arbitrarily so assumed for purposes of this calculation. On this assumption the following was secured:

TABLE I

	TUFTS ABSENT	TUFTS PRESENT
Closed core	71	65
Open core	45	34

In this four-fold table the alternative variates in both cases are unquestionably continuous. Testing first for goodness of "fit" (2), it was found that P was relatively small (0.000865); this being the case, the "tetrachoric r_t" was determined (4). For this table $r_t = -0.066 \pm 0.073$. This indicates that there is no correlation between openness of core and the phenomenon of tufts.

OPEN CALYX.—SORAUER is inclined to think that the fungus (as he calls it) which constitutes the tufts probably enters the apple through the calyx cavity at some time during the development of the fruit. Thinking that there might be some association or correlation between open type of calyx and tufts, the *Cyclopedia of Hardy Fruits* was again consulted, and 201 varieties were described definitely enough so that they could be tabulated for this correlation:

TABLE II

	TUFTS ABSENT	TUFTS PRESENT
Closed calyx	87	75
Open calyx	20	19

Calculating as described for table I, it was found that $P = 0.0053$, and that "tetrachoric r_t" $= -0.139 \pm 0.069$. This unquestionably indicates that there is no correlation between type of calyx and the presence of tufts.

ABORTIVE SEEDS.—It was thought that there might be some relation between tufts and abortive seed in an apple. From data secured by the writer, it is apparent that one of the factors which cause abortive seed is that when competition is strong enough between fruits, an apple will abort seeds something like a tree will drop fruit if the food supply is low or the tree is suffering from lack of moisture. If tufts are a fungal development, however, as stated by SORAUER, it seems logical to suppose that there might be some correlation between these phenomena.

Size of seeds in apples range from a plump mature seed down to a rudimentary type hardly discernible to the eye. In this paper "abortive seed" is one in which neither embryo nor endosperm has developed. In many seeds to determine this it was necessary to section the seed longitudinally. The seeds were cut in this way, since if only a small endosperm is developed it would be found at

the micropyle end of the seed. On this basis the data given in table III were secured.

In table III the x variate admits of only two divisions, which are certainly not discrete, as the amount of tufts is continuous from a maximum to the condition where no tufts are found. As one series consists of graduated measures and the other is dichotomous, the

TABLE III

CORRELATION BETWEEN ABORTIVE SEEDS AND TUFTS IN NORTHERN SPY; NUMBER OF FRUITS, 483; COEFFICIENT OF CORRELATION $r = -0.277 \pm 0.054$

CONDITION	ABORTIVE SEED												TOTAL
	0	1	2	3	4	5	6	7	8	9	10	11	
Tufts	18	28	26	30	34	28	16	7	3	0	1	2	193
No tufts	48	62	60	49	24	28	10	3	3	1	2	0	290

bi-serial r formula, as given by KELLEY (7), is used to determine the amount of relationship between the two characters under discussion. Considering the size of the sample and the amount of correlation determined, it appears that there is no significant relation between these two characters.

Relation between tufts and amount of seed for size of apple

In cutting a large number of apples for seed counts, it became apparent that there was some relation between number of seeds and the presence of tufts. In Northern Spy particularly was this noticed. In this variety, if one found a large fruit with lower than the average number of seeds for this sized apple, tufts were nearly always present. The same sized apple with a normal number of seeds, or a small apple with the same number of seeds, as a rule would show no tufts. To get some basis for making a correlation calculation, the weight of the seed was divided by the weight of the apple, which would give a ratio between these characters for each fruit. This would give a low ratio for apples having heavy weight and few seeds, and small or large apples having many seeds would have a high ratio. This again gives an array in which one series consists of graduated measures and the other is dichotomous. These

data are presented in table IV. Noting the correlation coefficient obtained from this table, it is evident that there is a considerable degree of correlation between low seed content for the size of the apple and the presence of tufts. From this it would follow either

TABLE IV

RATIO BETWEEN SEED AND FRUIT WEIGHTS; NUMBER OF FRUITS, 465; COEFFICIENT OF CORRELATION $r = 0.505 \pm 0.046$

Condition	3	5	7	9	11	13	15	17	19	Total
Tufts	28	68	55	32	13	.....	.....	.....	.....	196
No tufts	7	42	91	62	39	12	9	3	4	269

that if an apple has few seeds for its weight it has a surplus of material which goes into the production of tufts, or that since tufts are found along carpel fissures, when an apple of certain varieties has few seeds the carpel walls are more likely to crack, thus permitting tufts to develop.

Pathological studies

Since SORAUER considered that tufts are a fungus, comprehensive studies using pathological technique were employed in studying this phenomenon in apples.[1] While several varieties of apples were examined with the idea of determining the nature of apple tufts, considering the number of named varieties a representative number was by no means studied. Varieties studied included Northern Spy, Tallman, Delicious, Newtown, Baldwin, R. I. Greening, Jonathan, Ortley, York Imperial, and Ben Davis, in this and the following phase of this paper.

Working on the assumption that tufts are a fungus, attempts were made to cause them to grow and fruit in the way that fungi are usually studied. Portions of the carpel wall containing tufts were removed from the core of the apple, and placed in tubes containing apple agar made from the same variety of apple from which the tuft studied was secured. Fifty tubes streaked in various ways as to depth, amount of tuft, etc., failed to cause the tufts to develop to the slightest observable degree. Also portions of apple in Petri and

[1] This phase of the work was carried on with the assistance of Dr. E. REED of the Department of Botany at Syracuse University.

Stender's preparation dishes were autoclaved, and tufts placed in and on this material refused to develop. Receptacles containing tufts were placed in an incubator and subjected to a temperature of 40° C. without effect. Part of these were then removed to a room with a temperature of 5° C. Samples were subjected to these conditions from three to fifteen days, hoping that the changes from one extreme to another would shock the assumed fungal growth and cause it to fruit.

No set or combination of conditions caused the tuft to develop to the slightest extent. While the tufts were thus being studied they were examined daily with the microscope, and it was uniformly noted that the tufts were the same color and would dry out in the same manner as the apple tissue under all the conditions mentioned. Special fungal stains such as alum eosin were used, and in all cases the tuft took the stain the same as did the apple tissue. These results lead to the conclusion that apple tufts are not a fungus. At the same time, the tuft being very similar to apple tissue, except as to form, makes an excellent medium for fungi that develop in apples, so that when any part of a fungus comes in contact with a mass of tufts, in all likelihood it will develop under suitable temperature conditions, and this might lead one to think that the tufts were masses of fungal tissue. Such confusion of tuft and fungal tissue may explain the conclusions of SORAUER on the subject.

Microscopic studies

Coincident with the pathological work, areas of carpel wall were selected containing tufts attached to apple tissue. These were removed from the apple core, killed, and fixed by using Flemming's solution. Following the usual processes some of the material was imbedded in paraffin, but owing to the difficulty of cutting the tough leathery carpel wall it was necessary to use celloidin as an imbedding material. Various stains were tried, such as alcoholic safranin, iodine, light green, iron alum, and alum eosin, but none gave results comparable with a light stain of Kleinenburg's haematoxylin. With this stain the apple and tuft tissue take on a clear purplish-blue color and the carpel wall remains unstained, giving a marked contrast between the different types of tissue. The tufts take the stain in

the same amounts as to color as the apple tissue just outside the carpel wall, indicating that they are identical with apple tissue and are not fungi or storage bodies, as might be indicated by the correlation of tufts and low ratio between weight of fruit and weight of seed previously mentioned. The sectioned and stained material shows clearly that the tufts are proliferations which develop from the pith tissue in some varieties when a fissure forms in a carpel wall (figs. 5, 6). Frequently areas and sometimes single cells of the carpel wall are separated when the wall cracks, and these may be dislocated and pushed out by the developing mass of tufts (figs. 3, 4).

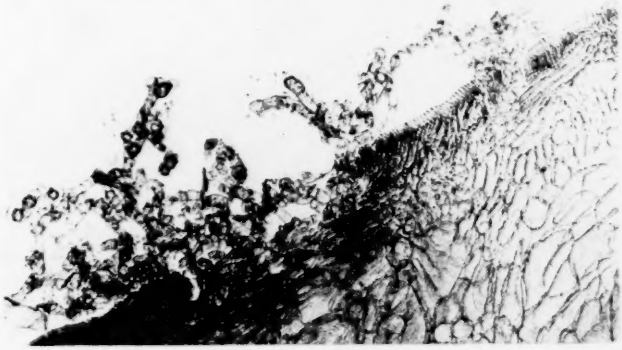

FIG. 5.—Newtown: cross-section showing tuft tissue through fissure in carpel wall and protuberances on strands of middle group; ×100.

While tufts are found on seeds, the sections made failed to show that at any place they were more than superficially attached to the seed. It seems probable that as they develop they grow along the surface of the seed where it happens to be contiguous to a carpel fissure.

It was noticed that there was a slight difference in the tufts produced in the different varieties of apples. In Northern Spy the cells in the strands average 65 μ in length and about 20 μ in diameter. Tallman shows cells slightly less in diameter, but of about the same length. The chief difference noted is in the terminal cells. Fresh material of Northern Spy shows protuberances stalked and almost globular which are iridescent under the microscope. Tall-

man shows these protuberances, but they are not so well developed as to size and show no coloring. Fresh material shows round bodies in the cells, some protoplasmic streaming, and with an iodine stain nuclei are quite distinct in the terminal cells.

Whether any physiological conditions are a factor in the development of tufts, and whether they are of economic significance have not been determined yet by the writer. It seems that their presence

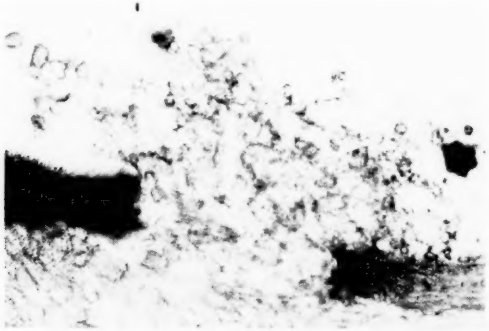

FIG. 6.—Newtown: cross-section of typical type of tuft; ×100

is a varietal characteristic, however, and one would be justified in using them as a means of classification of apple varieties if desired.

Summary

1. Tufts in apples are not fungi, but are tissue proliferations developed in carpel fissures in certain varieties of apples.

2. Tufts are found in high percentages in some of our longest keeping varieties, and while they may be a factor in decay, apples containing them will store without decay for long periods.

3. Tufts are not correlated with open core, open calyx, or with abortive seeds.

4. In Northern Spy tufts develop when an apple has a low seed content for its size.

5. Tufts produced in different varieties of apples show slight differences.

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EFFECT OF LIGHT ON GROWTH OF EXCISED ROOT TIPS UNDER STERILE CONDITIONS[1]

WILLIAM J. ROBBINS AND W. E. MANEVAL

(WITH TWO FIGURES)

Experiments on the growth of excised corn root tips under sterile conditions, reported in previous papers (**2, 3, 4**), showed that if the original excised root tip was grown in the dark for ten days or two weeks in Pfeffer's solution plus 2 per cent glucose, and its tip then cut off and transferred to a fresh solution at two-week intervals, the growth ceased in the third period. It was found that the addition of autolized yeast or peptone to the solution permitted the growth of the root tips to continue for as long as 4–6 periods in the dark. Even in the presence of autolized yeast or peptone, however, the rate of growth continually decreased as the periods passed, and eventually stopped. Attempts to improve conditions by increasing the concentration of autolized yeast showed that while 400 ppm of autolized yeast was somewhat more favorable than the other concentrations used, there was no marked difference between the effects of the various concentrations, and none of them permitted continued growth. TURNER (**6**) and KOTTE (**1**) have also grown excised root tips of higher plants under sterile conditions.

The specific problem dealt with in these investigations is the problem of defining the classes of materials which must be present, and the conditions that must exist, in order that the root cells will continue to grow. Do the cells of a corn root require something more than the mineral salts of Pfeffer's solution, oxygen, water, and glucose for their growth? The results of the experiments performed thus far and reported elsewhere could be interpreted as indicating that these materials are insufficient for the continued growth of excised roots, and that peptone or autolized yeast supply a part of the deficiency. Other interpretations of the data might

[1] Published with the permission of the Director of the Agricultural Experiment Station, University of Missouri.

be made, however, and other explanations offered for the cessation of growth to which attention has been called.

The present paper is the record of experiments in which the effect of a second factor favorable for the growth of excised corn roots is reported. This factor is light. Diffuse light favors the growth in Pfeffer's solution plus 2 per cent glucose of an excised root tip when its meristematic tissue is transferred to fresh solutions at intervals. In the presence of both light and autolized yeast, excised root tips of corn have been kept growing in Pfeffer's solution plus 2 per cent glucose for 149 days through ten periods of transfer.

In an earlier experiment, performed by the senior writer, and which has not been reported, the excised root tips of corn were grown under sterile conditions in culture flasks which were aerated by bubbling air through the solution continuously during the experiment. These flasks were placed in diffuse light to permit their attachment to a suction pump. It was noted that the growth of the root tips in both aerated and non-aerated cultures in the second growth period was superior to that in any of the experiments previously performed in the dark. An experiment was begun April 2, 1921, therefore, to test the effect of light and the combined effect of light and autolized yeast upon the growth of the excised root tips.

In experiment 13 the methods used were similar to those used in experiments previously reported. Root tips of Longfellow flint corn were grown under sterile conditions in 50 cc. of solution in 150 cc. Erlenmeyer flasks of Pyrex glass, a single root tip being placed in each flask. The solutions used consisted of the modified Pfeffer's solution plus 2 per cent glucose, and the same solution with 80 ppm or 400 ppm of autolized yeast added. The autolized yeast extract was prepared in essentially the same way as described earlier. Half of the flasks in each set were placed in diffuse light from a north window, and the other half were wrapped in black paper, covered with a black cloth, and placed in the same location. No method was available for controlling the temperature, which varied from 16° to 30° C. At two-week intervals the primary tip of each root was cut off and transferred to a fresh solution of the same composition as the one in which it had previously grown.

Three days after the root tips had been cut from the grains and transferred to the solutions, some of those in the light had developed anthocyan. Some of the roots in the light never developed anthocyan, however, and none of the roots in the dark developed this pigment. At the end of a week the distribution of anthocyan was very interesting. The heaviest development was in the older part of the root near the base, and around the points of emergence of the secondary roots. That portion of the root in which no secondary roots had yet developed showed red spots, indicating the points at which secondary roots were pushing through the cortex. The root cap cells also were red. The heaviest development of anthocyan occurred in the first period. There was apparently no positive correlation between the development of anthocyan and growth; in fact, the two roots which grew for the longest period contained no anthocyan.

During the first two weeks of growth there was little difference in gain in length (table I) between those roots which grew in the dark and those which grew in the light. A greater production of secondary roots in the dark was evident, both with and without the autolized yeast. The dry weight was somewhat greater in the light than in the dark. In the second period of growth, however, the difference between those roots grown in the light and those grown in the dark was very marked. Average length, secondary root production, and dry weight were distinctly greater in the light than in the dark. The difference was most marked in the set without autolized yeast. In the third period the roots in the dark in the modified Pfeffer's solution had ceased growth. In the fourth period those roots in the light in the Pfeffer's solution grew very little, and those in the dark in the solutions containing autolized yeast were discontinued because of the small amount of growth. In the fifth two-week period, six of the original eleven roots in the light in the modified Pfeffer's solution containing 80 ppm of yeast were still growing, and eight of the original ten roots in the light in the Pfeffer's solution containing 400 ppm of yeast were still growing. The number of roots decreased as continued transfers were made, due to contaminations or to the roots ceasing growth. Only two roots were left in the eighth period, one in the Pfeffer's solution

plus 80 ppm of yeast and one in the Pfeffer's solution plus 400 ppm of yeast. Both were transferred to solutions containing 400 ppm of autolized yeast and continued there. One of these became contaminated in the tenth period, and the other was accidentally discarded after growing nine days in the eleventh period, a total growth of 149 days under excised conditions. The individual records of these two roots are shown in table II. Figs. 1 and 2 represent graphically the average gain in length of the root tips grown under light and dark conditions in the Pfeffer's solution containing glucose, and in the same solution to which 400 ppm of autolized yeast were added.

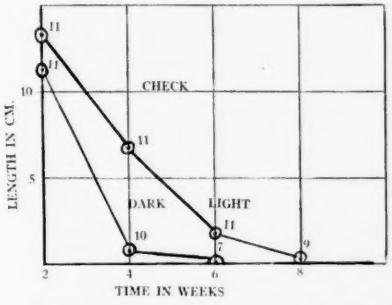

FIG. 1.—Average gain in length of excised corn root tips grown in modified Pfeffer's solution plus 2 per cent glucose in dark and in diffuse light; transfers of meristematic tissue made at two-week intervals; numbers on curves are number of root tips at each period.

In a second experiment (no. 21), begun December 11, 1922, root tips were grown in diffuse light both with and without autolized yeast. Again the beneficial effect of the light was evident. Instead of ceasing growth in the third period, six of the ten roots

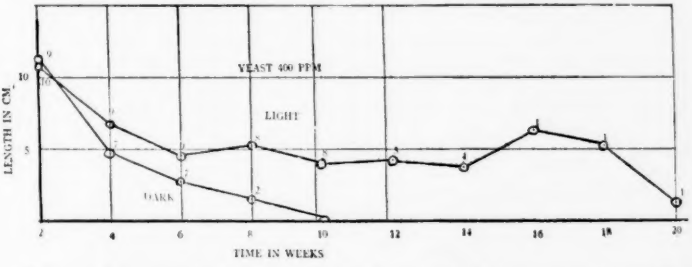

FIG. 2.—Average gain in length of excised root tips of corn grown in modified Pfeffer's solution plus 2 per cent glucose and 400 ppm of autolized yeast in dark and in diffused light; transfers of meristematic tissue made at two-week intervals; numbers on curves are number of root tips at each period.

grew in diffuse light through six periods of transfer in the Pfeffer's solution containing 2 per cent glucose. All but one of the ten

TABLE I

GAINS IN LENGTH, SECONDARY ROOT PRODUCTION, AND DRY WEIGHT WHEN GROWN IN DARK OR IN DIFFUSE LIGHT IN MODIFIED PFEFFER'S SOLUTION PLUS 2 PER CENT GLUCOSE WITH OR WITHOUT AUTOLIZED YEAST; TRANSFERS OF MERISTEMATIC TISSUE MADE TO FRESH SOLUTIONS AT TWO-WEEK INTERVALS

Addition to Pfeffer's solution plus 2 per cent glucose	Condition	Period	No. roots	Average original length (cm.)	Average gain in length (cm.)	Average no. secondary roots	Dry weight per 10 roots
None	Light	1: April 2–16	11	1.8	13.1	48	0.1118
None	Dark		11	1.7	11.4	87	0.0838
80 ppm yeast	Light		11	1.6	11.3	47	0.1094
	Dark		12	1.7	10.9	84	0.0913
400 ppm yeast	Light		10	1.7	10.9	51	0.1134
	Dark		9	1.4	11.1	72	0.1035
None	Light	2: April 16–30	11	2.1	6.9	19.0	0.0163
None	Dark		10	1.9	0.8	5.9	0.0030
80 ppm yeast	Light		9	1.6	8.7	33.0	0.0173
	Dark		9	2.0	3.8	7.0	0.0050
400 ppm yeast	Light		9	2.0	6.6	22.0	0.0129
	Dark		7	1.8	4.8	16.0	0.0060
None	Light	3: April 30–May 14	11	1.8	1.9	5.5	0.0073
	Dark		7	1.3	0	0.1	0.0020
80 ppm yeast	Light		9	2.0	4.1	7.7	0.0063
	Dark		9	1.7	0.8	0.5	0.0033
400 ppm yeast	Light		9	2.1	4.5	8.7	0.0062
	Dark		7	1.8	2.9	1.7	0.0020
None	Light	4: May 14–30	9	1.2	0.3	1.3	0.0042
	Dark		.....	.....	.....	.....	.....
80 ppm yeast	Light		7	1.6	3.5	5.0	0.0034
	Dark		4	1.7	0.1	0.0	.....
400 ppm yeast	Light		8	1.8	5.4	11.5	0.0082
	Dark		2	1.8	1.5	0	.....
80 ppm yeast	Light	5: May 30–June 13	6	1.6	1.5	3.5	0.0010
400 ppm yeast	Light		8	1.7	4.0	12.1	0.0035
80 ppm yeast	Light	6: June 13–27	5	1.3	2.7	1.8	0.0040
400 ppm yeast	Light		5	1.5	4.2	9.4	0.0090
80 ppm yeast	Light	7: June 27–July 11	3	1.6	2.9	4.6	0.0030
400 ppm yeast	Light		4	1.5	3.8	5.5	0.0170
80 ppm yeast	Light	8: July 11–25	2	1.7	2.1	3.5	.....
400 ppm yeast	Light		1	1.7	6.5	15.0	.....
80 ppm yeast	Light	9: July 25–August 9	1	1.6	6.5	4	.....
400 ppm yeast	Light		1	1.5	5.3	9	.....
80 ppm yeast	Light	10: Aug. 9–23	1	1.8	5.1	11	.....
400 ppm yeast	Light		1	1.3	1.2	1	.....

ceased growth in the sixth period. This single root, however, was grown for ten periods, when the experiment was discontinued. In

diffuse light eight of the thirty-seven roots were still growing in the same solution plus 400 ppm of autolized yeast after ten periods of transfer.

In a third experiment (no. 23), begun December 17, 1922, the modified Pfeffer's solution plus 2 per cent glucose was used. Grains of corn with the root attached were placed in one series of flasks, a single grain to a flask. Part of the flasks were exposed to diffuse light and part placed in the dark. Root tips were severed at the same time from other grains and transferred to flasks, part of which were placed in the light and part in the dark. After two weeks growth the tips were severed and transferred to fresh flasks. At

TABLE II

GAINS IN LENGTH AND SECONDARY ROOT PRODUCTION FOR TEN PERIODS OF TWO OLDEST ROOTS IN EXPERIMENT 13; NO. 57 CONTAMINATED IN TENTH PERIOD; NO. 34 ACCIDENTALLY DISCARDED AFTER GROWING NINE DAYS IN ELEVENTH PERIOD

		Period										Total
		1	2	3	4	5	6	7	8	9	10	
Root No. 57	Gain in length (cm.)	14.0	6.8	4.5	6.6	6.3	8.6	8.0	6.5	5.3	1.2	67.8
	Secondary roots	73	18	4	7	16	20	12	15	9	1	175
Root No. 34	Gain in length (cm.)	10.1	8.2	3.7	4.1	4.4	6.5	7.0	4.1	6.5	5.1	59.7
	Secondary roots	9	32	7	1	4	9	14	7	4	11	98

two-week intervals further transfers were made, until growth ceased. There was thus one set of root tips in the light and one in the dark which grew for the first two weeks attached to a corn grain, and a second series of root tips which grew detached from the grain from the beginning of the experiment. The results are given in table III.

The beneficial effect of the light was less marked in this experiment than in the other two. During the first period both those roots attached to the grains and those severed grew much better in the dark than in the light. In the second and third periods little difference was evident. After the third period, however, a distinctly beneficial effect of the light was evident, both in the amount of growth and in the number of roots which survived. In the case of those root tips grown in the excised condition from the beginning

of the experiment, six of the ten in the light were still growing, while none in the dark were growing after four transfers. For those roots which grew attached to a grain for the first two weeks, the beneficial effect of the light was less noticeable.

This experiment also shows another fact of interest. Those roots which grew attached to a grain during the first two weeks

TABLE III

GAINS IN LENGTH AND SECONDARY ROOT PRODUCTION WHEN GROWN UNDER EXCISED CONDITIONS FROM BEGINNING, AND WHEN GROWN FOR FIRST TWO WEEKS ATTACHED TO GRAIN, IN DARK AND IN DIFFUSE LIGHT

PERIOD	CONDITION	DETACHED FROM START			ATTACHED DURING FIRST PERIOD		
		No. roots	Average gain per root (cm.)	Average no. secondary roots	No. roots	Average gain per root (cm.)	Average no. secondary roots
1	Dark	16	10.25	50.0	13	26.7	108.0
	Light	15	5.6	7.7	8	13.3	72.0
2	Dark	11	2.2	9.0	10	6.2	30.6
	Light	13	3.65	7.8	6	4.1	3.0
3	Dark	11	0.38	0.9	10	1.46	4.8
	Light	12	1.6	3.1	6	2.1	3.5
4	Dark	1	1.6	0	9	1.4	1.0
	Light	10	2.4	4.4	5	2.4	3.2
5	Dark	1	1.6	0	2	3.2	1.5
	Light	8	2.2	4.2	5	2.4	2.6
6	Dark	.....	.....	.....	2	0.6	0
	Light	7	1.7	2.5	4	1.4	1.0
7	Dark	.....	.....	.....	1*	5.8	11
	Light	6	2.1	2.3	1	2.4	0
8	Dark	.....	.....	.....	1†	0.7	3.0
	Light	.....	.....	.....	.....	.....	.....

* Period was four weeks.
† Period was three weeks.

made a much greater growth in the second period than when they grew under excised conditions from the start. This beneficial effect is very evident in the third and fourth periods in the number of roots which survived, and is also evident in the fifth and sixth periods. In fact, one root tip grew a total of 133 days in the dark, 119 days under excised conditions. This is the longest period in which an excised corn root tip has been grown in the dark in the modified Pfeffer's solution.

Discussion

While diffuse light has a beneficial effect upon the growth of excised root tips of corn under the conditions of the experiments, the effect is not great enough to permit continued growth. In the later periods the roots become thin and threadlike, the elaboration of dry matter is slight, and the roots cease growth. The cause of the beneficial effect of the light is problematical. Does it aid in the elaboration of some accessory food substance, assist in the utilization of some material already present in the solution, or reduce the injurious action of some factor or factors which slowly cause the root tips to cease growth? The determination of the reason for the cessation of growth of the root tips will probably explain also the beneficial effect of the diffuse light. It is not due to the production of chlorophyll in the roots, although SIEBERT (5) has reported the development of chlorophyll in the roots of several plants in the light.

The beneficial effect on growth in later periods of permitting a root tip to remain attached to the grain during the first two weeks might be interpreted as substantiating the hypothesis that an accessory food substance is secured from the grain. After the root tip is separated from the grain it would depend upon the quantity of accessory material contained in it, and therefore the growth in the second period of those roots attached to the grain during the first period is much like the first period growth of the root tips excised from the start, and in the third period like that of the second period growth of the roots excised from the start. On the other hand, we might also assume that the stoppage of growth is due to the cumulative effect of some injurious factor, which is reduced because of the greater proportion of tissue present in the case of those roots which grew attached to the grain during the first period.

Summary

1. Diffuse light was favorable to the continued growth of root tips in a modified Pfeffer's solution containing 2 per cent glucose, or the same solution to which 80 ppm or 400 ppm of autolized yeast were added.

2. Anthocyan developed in some of the excised corn roots in the light, but in none in the dark.

3. The anthocyan pigment was present in the root cap, at points where secondary roots were to emerge or had emerged, and at the base of the root, but not in the meristematic region.

4. The longest period a root tip was grown was 149 days, through ten periods of transfer, in diffuse light in the modified Pfeffer's solution containing 2 per cent glucose and 400 ppm of autolized yeast.

5. Roots which remained attached to the grain during the first two weeks grew for a longer time than those grown under excised conditions from the start. One such root was grown in the dark for 133 days, 119 in excised condition.

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SECONDARY STELAR STRUCTURES OF YUCCA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY **327**

GRACE BARKLEY

(WITH NINE FIGURES)

Yucca belongs to a group of monocotyledons peculiar in having a cambial ring producing secondary tissues which increase the size of the stem as do the secondary tissues of dicotyledons and gymnosperms. These secondary tissues are very characteristic of this group, since they differ in important features from those of gymnosperms and dicotyledons.

DEBARY[1] called attention to the secondary growth in the arborescent stems of *Dracaena*, *Yucca*, *Aloe*, and others, and described the group in general. He stated that the initial layer of cambium consisted of and remained a simple layer of cells, and that it must be regarded as belonging to the plerome cylinder. He also described the secondary tissues of *Dracaena*. SCHOUTE[2] stated in regard to this group that at first the cambial layer is regenerated at the expense of the cortical layer external to it, and later a definite initial layer is differentiated. He made some measurements of the thickness of the growth from the cambium, and tabulated the measurements of the primary and secondary cylinders of several of these stems, doing more work on *Dracaena*, but also including *Cordyline rubra* and *Yucca filamentosa*. CHAMBERLAIN[3] announced the discovery of growth rings in the secondary tissues of *Aloe ferox*. He also described the primary and secondary bundles of this stem.

Most of the arborescent monocotyledons, like the herbaceous forms, do not have secondary growth. The diameter of their stems is determined by growth in the primary tissues and by the differentiation of these primary tissues, after which no further change

[1] DEBARY, A., Comparative anatomy of the Phanerogams. 1884.

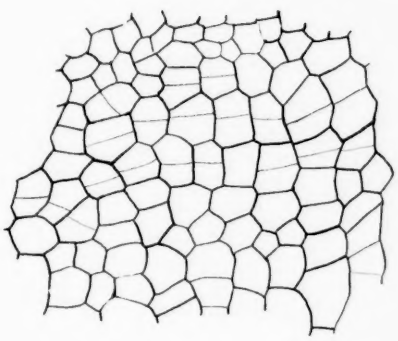
[2] SCHOUTE, J. C., Die Stammesbildung der Monocotylen. Flora **92**:32–48. 1903.

[3] CHAMBERLAIN, C. J., Growth rings in a Monocotyl. BOT. GAZ. **72**:293–304. 1921.

takes place. Like other monocots, *Yucca aloifolia* has a primary cylinder in which the bundles are scattered through the parenchyma. This cylinder is surrounded by the cylinder of secondary tissues composed of bundles scattered through parenchyma. The secondary cylinder is hard, and in the stem studied showed growth rings which, to the naked eye, resembled the growth rings of woody dicotyledons. This cylinder is surrounded by the primary cortex, which is inclosed by the secondary cortex.

The cambium ring first makes its appearance 1.5 cm. from the apex of the stem, originating in the pericycle. Several rows of cells begin to divide (fig. 1), making the initial cambium a zone of meristematic tissue. The young cambial cells have the radial diameter about one-third the other two dimensions, which are about equal. New tissue is produced on the inner side and becomes several cells thick before differentiation begins. The tissue produced by the cambium becomes differentiated into secondary bundles and parenchyma. A cell in one of the radial series from the cambium begins to divide radially and tangentially at first (fig. 2), and then in all directions, until numerous cells are formed. One or two adjacent cells from adjacent series may begin division at the same time and combine in forming a bundle, so that a bundle may be formed from one or two mother cells from one or two radial rows.

In the stem studied there were very distinct growth rings. These were formed by an increase in the number of vascular bundles, which are more numerous there than in the region between the rings (fig. 7). Generally there are three or four rows of parenchyma cells between the bundles of the same row in the rings, and about twice as many cells between the rows of bundles. The bundles are further separated in the region between the rings, and they are also further separated in a radial direction, the rows of bundles being about twice as far apart as in the rings. The bundles between the rings are a little larger than those in the more crowded region of the ring. The parenchyma becomes lignified in the growth rings, reaching a greater lignification in the older rings, and remains unlignified in the region between the rings. The secondary bundles are soon differentiated into xylem and phloem. The phloem lies toward the periphery of the stem and is generally on the outside of



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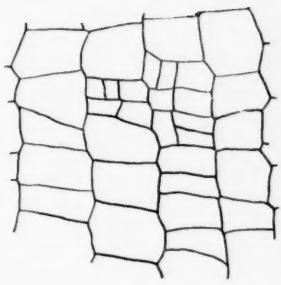

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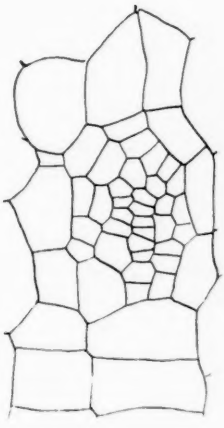

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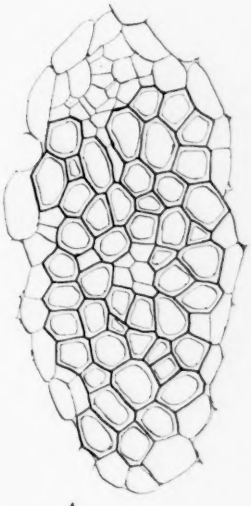

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FIGS. 1–4.—Fig. 1, initial cambium in several rows of cells; fig. 2, young bundle beginning with several mother cells; fig. 3, young bundle which originated in one radial row; fig. 4, usual secondary bundle.

the bundle. In some bundles the xylem almost surrounds the phloem, and sometimes a completely amphivasal condition is reached (fig. 5). This takes place where the bundles are more crowded.

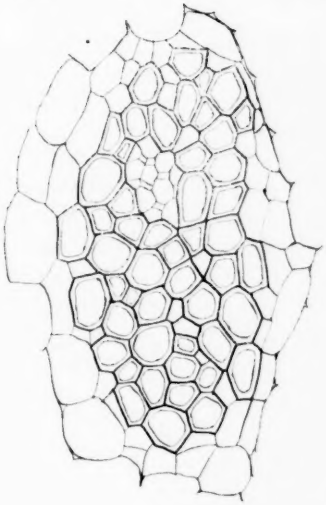
The xylem is composed of tracheids which have small, narrow, oblique bordered pits arranged in one or two rows on their radial and tangential walls, and a few thin walled parenchyma cells. The phloem is very small, and, in transverse section, shows six to eight sieve tubes with companion cells.

The secondary parenchyma is composed of cells usually longer than wide, with large intercellular spaces. All the cells have numerous round pits in their walls (fig. 6). The cells adjacent to the bundles have walls more thickened than the other cells, and this makes the pits more plainly visible in these regions.

Sections of wounds were studied and the secondary growth noted here also. Two cambiums are produced in these wounds parallel to the face of the wound, a cork cambium and an inner cambium. The tissue produced on the inner side of the inner cambium is differentiated into bundles and parenchyma, as is that produced by the cambium of the stem. Considerable callus was built on the face of the wound before the cambium formed, and this is formed on the inner side of the callus near the bundles in the old tissue. These wound bundles are generally like the secondaries of the stem, with about the same amount of phloem and xylem. The phloem is always toward the cambium, and this makes the new bundle take various positions relative to the old bundles.

In some places the new bundles are suberized, and the lumen of the cells becomes filled as soon as the cells become differentiated. A sheath of parenchyma is also formed about these bundles, which becomes suberized. The intervening parenchyma remains unmodified. In most places only the outer part of the new cortex is suberized.

A peculiar modification of the new bundle was found in a place where they were formed under the influence of two wounds. These wounds were perpendicular to each other, and the end of one was 0.25 inch from the face of the other. Two rows of new bundles were formed parallel to the face of one of the wounds, but the



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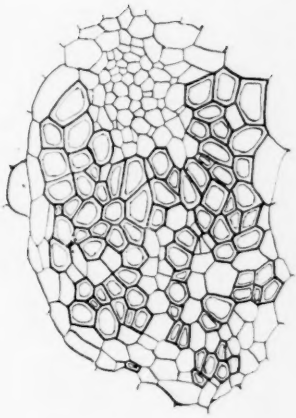

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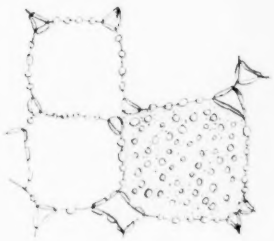

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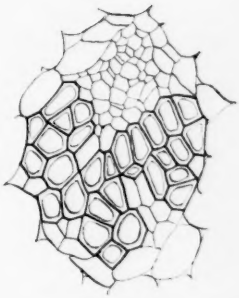

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FIGS. 5–9.—Fig. 5, amphivasal secondary bundle; fig. 6, radial section of parenchyma showing pits in walls; fig. 7, diagram showing distribution of secondary bundles in growth rings; fig. 8, larger primary bundle; fig. 9, smaller primary bundle.

phloem, instead of being on the side of the bundle toward the cambium that formed the bundle, was toward the other wound.

There are two kinds of primary bundles, one being larger than the other. The larger bundle, in transverse section, shows a small amount of phloem, about 20–30 sieve tubes and companion cells (fig. 8), located on the outer side of the bundle. The xylem of this bundle is composed of a few pitted vessels near the phloem, many tracheids with narrow oblique bordered pits, spiral vessels, and thin walled parenchyma. The smaller bundle, in similar section, shows less phloem, having about 10–20 sieve tubes and companion cells (fig. 9). The xylem has no pitted vessels and no protoxylem, and is composed of only the tracheids and parenchyma similar to those of the larger bundle.

The larger primary bundles are leaf traces, and they remain unchanged when they enter the stem, except that they lose the sheath. They have a cambium in the leaf base, which may be seen in the stem as far as 1.5 cm. from the end.

The leaf traces enter the primary cylinder and pass downward at an angle of about 25° toward the center of the stem, where they curve obliquely around the center and then pass downward on the opposite side, parallel to the axis of the stem. After secondary tissue is formed they pass through it horizontally until the primary cylinder is reached.

The material for this study was obtained from plants growing out of doors in the Gulf States, and from one of the greenhouses of the University of Chicago. The plant used in the study of the growth rings came from the greenhouse. It was about seven years old and was about 12 feet high, but it had been topped once. The main stem was 4 feet in length from the ground to the branches. It was 5 inches in diameter, with a 2 inch primary cylinder. The larger stems of the material from the Gulf States also showed growth rings.

It would be interesting to know to what extent growth rings are present in *Yucca* stems in their native habitat.

Summary

1. The cambium originates in the pericycle about 1.5 cm. from the end of the stem, and the initial cambium is a zone of meristematic tissue.

2. The secondary bundle originates from one or two cells in one or two radial series. They are usually collateral, but in some bundles the amphivasal condition is reached.

3. There were growth rings in the stems studied, formed by an increase in the number of bundles in alternating regions together with lignification of the parenchyma in these regions.

4. In the wound tissue a cambium is formed which produces a secondary tissue similar to the secondary tissue of the stem, except that some of the bundles are suberized, and these are surrounded by a sheath which is also suberized.

5. The leaf traces pass down the primary cylinder at an angle of about 25°, curve around the center of the cylinder, and continue on the opposite side parallel to the axis of the stem.

I am indebted to Professor CHARLES J. CHAMBERLAIN for suggestions and direction in this study.

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GROWTH-INHIBITING AND GROWTH-STIMULATING SUBSTANCES

W. F. Gericke

When pots containing fertile soils are seeded to wheat or barley for continuous seasons, and successive short period crops of four to six weeks' growth are removed from such soils, it is found that the first crop is usually the largest, the immediately succeeding crops are much smaller, but some of the latter crops may be larger than some of the preceding ones. Thus instead of soils becoming progressively poorer by continuous cropping due to progressive diminution of its supply of plant food, they may become better at certain times than they were before, through certain processes and agencies concerned in the growth of plants. The results obtained with soils (**7**) led the writer to conduct experiments with culture solutions, and to employ a method that would provide a number of successively grown crops under the same climatic complex.

As the method required that the same nutrient solution be used repeatedly, large glass bottles of 6 liter capacity were used as the culture containers. These were filled with a nutrient solution of two atmospheres osmotic value, which was composed of equal partial molar concentrations of KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$. The experiment was begun by planting a set of these containers with wheat seedlings. The plants were allowed to grow six weeks. They were then removed, and newly germinated seedlings, 4–6 cm. high, were placed in this set of containers. At the same time another set of containers having the original nutrient solution were likewise planted with wheat seedlings. The first set of containers, therefore, grew its second crop at the same time that the second set grew its first crop. Thus any number of crops grown successively and under the same climatic environment can be obtained by introducing a new set of culture containers into a series when the other sets thereof are replanted for progressive numbers of crops.

No addition of salts or renewal of nutrient solution was made during the course of the investigation, except to add distilled water

to the containers to replace loss by transpiration. At frequent intervals small quantities of $FeSO_4$ were added to the cultures to meet the demands of the plants for iron. Qualitative tests made at intervals during the investigation always showed a sufficient supply of nutritive elements in the culture solution. At the end of the experiment, the set of bottles which contained the nutrient solution that had supported seven crops still contained sufficient supply of salts to have supported several more crops. Some of the results obtained from this investigation are given in table I.

TABLE I

DRY WEIGHT IN GM. OF SUCCESSIVE CROPS GROWN CONTEMPORANEOUSLY AND UNDER SAME CLIMATIC CONDITIONS; AVERAGE OF FOUR CULTURES PER SET

Series no.	First	Second	Third	Fourth	Fifth	Sixth	Seventh
7	6.0±.07*	3.5±.27	4.4±.01	5.5±.06	4.9±.20	4.2±.20	4.6±.10
6	4.8±.23	3.4±.10	4.7±.10	3.8±.13	4.6±.13	4.4±.27	.....
5	3.4±.03	2.8±.02	3.4±.13	3.0±.10	2.6±.02	.....	.....
4	2.0±.13	2.7±.10	1.7±.10	2.8±.14	.....	.....	.....
3	3.3±.10	2.5±.02	3.3±.02	.....	.....	.....	.....
2	1.6±.00	1.5±.01	.....	.....	.....	.....	.....
1	1.7±.10	.....	.....	.....	.....	.....	.....

* Two cultures.

The "first crop" column gives the dry matter produced from wheat cultures grown in seven different series of containers filled with the original nutrient solution. They are crops grown under different climatic conditions. These first crop cultures followed one after the other in sequence of time, and there was no intermission between the harvesting of one crop and the replanting of new media with new seedlings. The growth period of each series was six weeks, except that of 1 and 2, which was one month.

All crops of any series were grown under the same climatic conditions, the same length of time, and in the same kind of nutrient solution, except in respect to such changes as have occurred incident to, or as the result of, the crops it had grown. Referring now to the crops of series 7, it is to be noted that the first crop was the largest and the second was the smallest of the series. All succeeding crops were markedly larger than the second. The depression in yield of the second crop must be accounted for in the properties of the

nutrient solution derived from, or being, the effects of the first crop. That is, either toxic substances have been produced by the first crop, or the decreased yield of the second crop was due to a diminution in the supply of plant food removed in the first crop. Due to the fact that succeeding crops did not show corresponding decreases in yield as a result of obvious diminution of nutritive elements in the culture media, however, it follows that the explanation must be sought in the first premise.

The results in series 6, 5, and 3 are similar to those of series 7, that is, a depression of yield follows the first crop and the third crop shows marked improvement over the second. Series 4 gave results somewhat different from the others, in that the second crop was the largest and the third crop was the smallest; but, similar to the results in all the other series, increased yield ultimately followed a low yield.

Comparisons between the yield of the second crop and the third crop of series 7, 6, 5, and 3, and between the third crop and the fourth crop of series 4, show that the average of the yields of the succeeding crops in question is approximately 36 per cent greater than the immediately preceding one. It appears from these data that the harmful effects due to the growth of the first crop, as evidenced in the depression of yield in the second (or third in series 4), did not exist for the third crop. The average yield of the third crop for all comparable series was only 8 per cent less than that of the first. In some cases, the yield of a succeeding crop was equal to that of the first. While the media in which the plants of the second crop were rooted produced markedly inferior plants, nevertheless the reaction and effect of the second crop on these solutions, which became the growth media for the third crop, were decidedly beneficial. That is, it seems that a growth stimulating substance was produced by the plants in the second crop, which benefited the seedlings placed in these solutions and accounted for the improvement in yield of the third crop. On the other hand, it may be argued that the harmful properties due to the first crop had been made innocuous or nearly so by the second crop, and therefore the improvement in yield of the third crop was not due necessarily to the production of growth stimulating substances by the second.

That growth inhibiting substances may be produced by plants has been suggested by some of the early botanists (5) as an explanation for certain ecological distributions of plants. Later, when critical study was given to problems of soil fertility and crop rotation, this matter was given more serious consideration. Through extensive investigations in this field, LIVINGSTON, BRITTON, and REID (9), LIVINGSTON (8), SCHREINER and REED (11), SCHREINER and SHOREY (10), and others came to the conclusion that soils may be infertile because of the presence of some substance or substances produced by the plants or resulting from the decay of vegetation that may be inimical to crop production. Investigations on the effect of one crop on another by BEDFORD and PICKERING (1) show that one plant may affect other plants deleteriously. The conclusions arrived at by these investigators are in part as follows:

(1) Every growing crop results in the formation of a substance which is toxic to the growth of other plants and still more to itself.

(2) By oxidation, these toxins lose their toxic properties and enhance the fertility of the soil. The plants previously poisoned eventually outstrip those which had not been subjected to the poisoning, except in cases where the toxic effect has been sufficient to produce stunting.

(3) The toxic effect must necessarily vary considerably with the different conditions, both as to the nature of the soil, the plant affected, and the vigor of growth of the plant producing the toxin.

(4) There is no reason for assuming the excretion of any toxic matter from a plant, the débris from the growing roots is probably sufficient to account for the formation of the toxin.

Field experiments conducted by FLETCHER (6) at Gizeh, Egypt, also bear out the conclusions relative to the deleterious effect of one crop upon another. These experiments were especially designed to test these points, as FLETCHER had conducted certain water culture experiments which led him to the conclusion that plants excrete toxic substances.

That organic plant constituents added to the culture media may have beneficial effects on vegetation has been shown by several investigators. Among these, the workers in the Department of Agriculture already referred to have shown that these beneficial effects may be accounted for by (1) rendering toxic substances innocuous, (2) utilization as nutrients. That toxic material upon

oxidation may prove decidedly beneficial to plants is the conclusion arrived at by BEDFORD and PICKERING, in the investigations already referred to. The investigations by BOTTOMLEY (**2, 3, 4**) with growth producing substances (auximones) derived from bacterized peat show that not only may the products of organic growth be beneficial to plants, but they may be essential and indispensable. Furthermore, as minute quantities of this material appear to suffice, it appears that the rôle this material plays in plant nutrition must be considered in a different light from that ordinarily accorded to the essential mineral elements.

The growth of plants is not only a complex of many processes, but is in a sense the result of the succession of processes. Some of these processes are dependent on certain salts which supply necessary inorganic elements to plants. In order that the plant may have these elements when needed, it is essential that there be sufficient supply in the culture media to meet the demands of the plant. The question may be asked in this connection, Are subsequent stages of growth dependent only on certain inorganic elements in media in the sense that these would supply the salt requirement for any particular growth phase? May not small amounts of substances of unknown composition arising from previous or contemporaneous growth processes of plants be essential for subsequent phases of growth in the same sense as are mineral elements in the culture media? Apparently the final answer to this question is not yet at hand.

The fact that plants may change the reaction of a culture media from a less to a more favorable condition is at least suggestive of an affirmative potentiality of this query. Furthermore, experimental data show that a constantly maintained supply of nutrients in culture media is not as favorable to the growth (entire life cycle) of cereal plants as is a changing one. This may indicate that any subsequent stage of growth may be beneficially affected by secretions from previous growth phases. That is, plants may be similar to animals in that they have a physiology which is subject to growth inhibiting, growth stimulating, and sensitizing agencies produced by the organism itself.

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INHERITED DEFICIENCY IN CARBOHYDRATE METABOLISM IN MAIZE[1]

WILLIAM H. EYSTER

(WITH THREE FIGURES)

When commercial varieties of maize are inbred, a relatively large percentage of them give progenies that segregate one or more types of chlorotic seedlings. The chlorotic conditions are due to genetic factors which in some way influence the development of the chloroplastid pigments. Albino plants are produced when the chloroplastid pigments are entirely lacking, although in some types a small amount of the pigments is developed, especially at the leaf tips and along the veins, so that they become cream colored. The life of such plants is limited to the food reserve in the endosperm, as they are unable to synthesize carbohydrates. A few types of albinos have been found, which, under the most favorable conditions, develop the chloroplastid pigments sufficiently to enable them to grow to a mature, although very weak plant, as illustrated by *virescent* white.[2]

Yellow seedlings are produced when there is a partial or complete development of carotin and xanthophyl, with only a slight development or complete absence of one or both of the chlorophyls (chlorophyl *a* and chlorophyl *b*). When the chlorophyls are entirely lacking, the yellow seedlings vary in color from cream-yellow to bright yellow, according to the amount of yellow pigment developed. The deep yellow types usually have small amounts of one or both of the chlorophyls, in addition to carotin and xanthophyl. Genetic types of maize have been found also which range from deep yellow, through various shades of yellow-golden, green, and olive green, to full green, according to the relative amounts of the different chloroplastid pigments produced.

[1] The investigation reported in this paper was made in connection with genetic studies of maize in the Department of Field Crops, Missouri Agricultural Experiment Station.

[2] LINDSTROM, E. W., Chlorophyl inheritance in maize. Cornell Univ. Agric. Exp. Sta. Mem. **13**: 1–68. 1918.

Preliminary studies[3] of a quantitative nature indicate that yellow seedlings which develop carotin and xanthophyl, but lack both chlorophyl *a* and chlorophyl *b*, are not able to synthesize carbohydrates. Types were found which contained (1) carotin, xanthophyl, and chlorophyl *a*, but lacked chlorophyl *b;* (2) carotin, xanthophyl, and chlorophyl *b*, but lacked chlorophyl *a;* and (3) carotin, xanthophyl, chlorophyl *a*, and chlorophyl *b* in varying amounts. So far as studies have been made, plants which lack either chlorophyl *a* or chlorophyl *b* are unable to synthesize sufficient carbohydrates to grow to maturity, but it was interesting to find that the two chlorophyls appear to be of unequal importance, as indicated by the length of time the different types were able to live after the food reserve of the endosperm was exhausted. The time between the complete digestion of the endosperm and death may be said, in general, to increase with the amount of the chlorophyls that develop in the chloroplastids, until they are sufficiently developed to enable the plant to synthesize the carbohydrates necessary to grow to a mature though very weak plant. Vigor increases as chlorophyl content approaches that of a normal green plant, although it is not necessary to have a full complement of the green pigments to produce a plant that makes an apparently normal growth, as is well illustrated by *golden* (LINDSTROM, 1918).

In all the genetic types mentioned, the inherited characters are concerned with the development of the chloroplastid pigments. All of them are able to utilize the carbohydrates stored in the endosperm, and live normally while they last. In the types which develop the chloroplastid pigments in sufficient amounts, life is prolonged according to the ability of the chloroplastids to synthesize carbohydrates.

In the summer of 1920 I found in cultures of maize a yellow seedling which differs from other chlorotic seedlings, in that it is unable to utilize the carbohydrates in the endosperm. Under field conditions this type of yellow seedling usually dies within a week after the appearance of the first leaf, while under more favorable

[3] The writer is indebted to the Bussey Institution of Harvard University, and especially to Dr. E. M. EAST, for facilities for making quantitative studies of the chloroplastid pigments in various genetic types of maize.

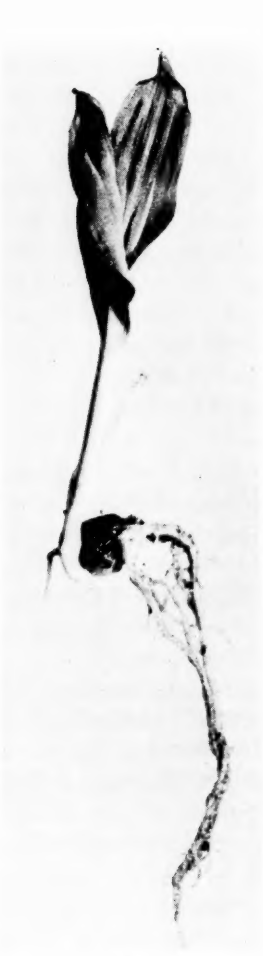

FIG. 1

FIG. 2

FIGS. 1, 2.—Maize seedling showing evidences of glucostacty less than a day after appearance of first leaf; fig. 2, glucostactous maize seedling with first leaf badly wilted, and second and third leaves partly autolyzed; evidences of glucose on ends of first and second leaves.

conditions in the greenhouse it has been kept alive for a longer period. The plant is deficient in the chloroplastid pigments, as shown by its yellow color. That the plant's deficiency extends beyond the development of the chloroplastid pigments and the synthesis of carbohydrates is indicated by the early autolysis and death of the young leaves. Autolysis begins almost as soon as the leaves are unrolled. The tips of the young leaves appear water soaked, and this condition rapidly extends down toward the base of the leaf. Soon the leaves appear wilted, and exude a liquid which at first is clear, but soon changes to a light brown and finally a dark brown color. Plants in this condition were observed to attract flies, and, upon tasting, the liquid was found to be quite sweet. This brown, sweet tasting liquid was analyzed[4] and found to be glucose with a trace of maltose. It was interesting to find that not even a trace of fructose was present.

FIG. 3.—Glucostactous maize seedling illustrating condition typical of plants at end of about a week after appearance of first leaf.

The plant illustrated in fig. 1 was photographed less than a day after the appearance of the first leaf. It may be seen that the tips of the first and second leaves show evidences of autolysis. Fig. 2

[4] The analysis was made by Dr. M. M. ELLIS, University of Missouri, by the osazone method.

shows the first leaf completely wilted, while the second and third leaves are partly autolyzed. There are evidences also of the brown liquid on the ends of the leaves. The plant shown in fig. 3 had all

TABLE I

F_2 PROGENIES FROM CROSSES EQUIVALENT TO *normal*×*glucostactous*

PEDIGREE	TYPE OF SEEDLING	
	Normal	Glucostactous
1088– 2(X)	105	27
– 3(X)	101	31
– 4(X)	61	18
–10(X)	68	24
–13(X)	88	27
–14(X)	107	27
–15(X)	71	43
–18(X)	58	19
1089– 1(X)	74	24
– 3(X)	105	37
– 5(X)	78	25
– 7(X)	85	32
– 9(X)	67	27
1091– 7(X)	101	47
1093– 4(X)	65	32
1094–18(X)	98	35
1099–12(X)	61	26
1572– 5(X)	107	36
–11(X)	31	19
–12(X)	57	18
–25(X)	58	11
–32(X)	30	15
–39(X)	43	17
–41(X)	59	17
–46(X)	16	5
–47(X)	37	7
–50(X)	33	13
–52(X)	20	5
1586–13(X)	113	31
– 8(X)	141	44
–12(X)	135	48
340– 4(X)	123	46
675– 8(X)	83	37
675– 5(X)	234	75
674– 1(X)	55	25
673– 4(X)	192	77
673– 1(X)	252	99
674– 2(X)	18	4
5055– 3(X)	264	64
Total	3494	1214
Expected (3:1)	3531	1177
Deviation	37 ± 63.3	

of the leaves badly wilted in less than four days after the appearance of the first leaf. These plants have all the appearances of being affected by a wilt. Attempts were made to inoculate other plants, with negative results. The roots were found to be perfectly normal as compared with the roots of the green plants of the same progenies.

The death of the yellow seedlings just described is not due to a lack of carbohydrates, but rather to an inability to utilize carbohydrates that are available, and are even present in the tissues in soluble form. The presence of large quantities of glucose in the

TABLE II

F_3 PROGENIES OF CROSSES EQUIVALENT TO *normal*×*glucostactous*

Pedigree	Progenies all green	Progenies segregated *normal* and *glucostactous*
1088	5	8
1089	0	6
1090	1	0
1091	5	1
1092	1	0
1093	1	1
1094	2	1
1099	0	1
1572	6	12
1586	0	4
Total	21	34
Expected (1:2)	18	37
Deviation	+3	−3

tissues of the dying seedlings indicates that the carbohydrates stored in the endosperm are digested and absorbed, but are not utilized in the plant's vital processes. As a result, the glucose accumulates and finally is exuded from the tips of the leaves to form drops. The physiological condition in plants represented by these sugar-sweating maize seedlings may be called *glucostacty*.

This inability of the plant to utilize the sugars that are already absorbed is strikingly like the diabetic condition in animals. Whether the similarity is real, or only apparent, must be determined by further physiological study.

Glucostacty, which represents a profound disturbance in the carbohydrate metabolism, is inherited as a simple Mendelian

recessive character, as is shown by the data recorded in tables I and II. The symbol *Gl* will be used in referring to the gene responsible for this deficiency in carbohydrate metabolism. In table I are listed F_2 progenies with a total of 4708 plants, of which 3494 were green and 1214 were yellow and unable to utilize their absorbed sugars. This is a deviation of 37 ± 63.3 from the numbers expected for a 3:1 ratio. In table II are listed 55 F_3 progenies, of which 21 consisted of all normal green plants, and 34 segregated green and glucostactous seedlings as shown in table I. This is a deviation of about 3 plants from the 1:2 relation expected.

Summary

Of common occurrence are inherited chlorotic types of maize which are partially or wholly deficient in one or more of the chloroplastid pigments. The first source of carbohydrates for maize seedlings is the endosperm. When this supply is exhausted the plant dies, unless it is able to synthesize additional carbohydrates sufficient to maintain life. A genetic type of maize has been found and described that digests and absorbs the endosperm, but is unable to utilize the sugars thus obtained in the vital processes. This deficiency in carbohydrate metabolism has been called glucostacty, and is inherited as a simple Mendelian recessive character.

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TYLOSES IN MENISPERMUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 328

LYNETTE MYERS

(WITH PLATES XI, XII)

Tyloses were found as long ago as 1675, when MALPIGHI (8) found ingrowths in the spiral vessels of the oak which to him resembled the air tubes of insects. Since then many have investigated their distribution, origin, behavior, and function.

Probably all investigators have found tyloses in many woody angiosperms, some of which have more abundant formations than others. GERRY (4) made a report on many American woods; MOLISCH (9) examined several hundred vascular plants, and decided that tyloses are not formed in vascular cryptogams and gymnosperms. WILLIAMSON (15) and WEISS (14) found them in the fossil fern *Rachiopteris*. WEISS stated that it would be impossible for tyloses to be formed in gymnosperms, as these plants do not usually have parenchyma; but in 1908 CHRYSLER (2) investigated some gymnosperms and found tyloses in them.

For more than a century, tyloses were considered swellings of the inner walls of the tracheae. In 1845 SCHLEIDEN (11) observed that they enter the vessels from the parenchyma, but this was disputed by BOEHM (1) and UNGER (13). UNGER claimed tyloses to be bulges of the inner layer of the vessels, but BOEHM believed them to be formed by a collection of plasma between the lamellae of the vessel walls, the inner layer growing into the tylose. REES (10) proved these views wrong, including figures showing tyloses entering the vessels. Later investigators (3, 6, 8) agree that tyloses originate from adjacent parenchyma cells, entering tracheae through their pits.

Growth of tyloses has been observed by many investigators, and their division was noted as early as 1847 (12). DE BARY (3), describing growth changes, states that the protrusion, at first short, blunt, and cylindrical, expands into a round and sometimes volumi-

nous bladder containing protoplasm and usually a well defined nucleus, and finally separates from the cell which produced it by forming a wall at its point of entrance into the vessel.

Functions of tyloses have been observed as follows. (1) They close the ends of conducting tubes that have been injured, as shown by their extensive formation at ends of cuttings and beneath scars left from broken branches. In both situations their number and growth are so great that they become flattened from contact pressure (**1, 5, 6, 13**). (2) The sclerotic tyloses add to the mechanical strength of the heartwood; in winter, some tyloses are receptacles for starch (**5, 6**); when starch is present, tyloses may assist in conduction by increasing the surface of contact between the vessels and the parenchyma cells; by acting in the capacity of haustoria, they may increase the exudation pressure, excrete sugar, or withdraw certain sap constituents.

Material and methods

The material used for the study of *Menispermum* was collected from the forests adjacent to the Des Plaines River, Cook County, Illinois. Specimens varied from 1 m. to 8 mm. in diameter, and were taken at intervals of six inches from ground to tip, a distance of ten or twelve feet. These parts of the stem were fixed in a solution of formalin, alcohol, and acetic acid as soon as collected, the fixing solution being taken to the place of collection, that no change might take place in the material.

Some very good results came from sections of the fresh materials. Probably the best results were obtained from freehand sections stained with safranin and Grubler's light green, and cleared with clove oil, but paraffin sections were also used and they showed some details very clearly. Cellulose acetate was tried, but it does not soften this wood sufficiently to yield smooth paraffin sections.

Investigation

A preliminary study was made during the summer of 1920, and tyloses were found to be very abundant. The tracheae near the pith in most cases were filled with these growths (fig. 1), but vessels near the outer part of the stem did not contain any. These tyloses,

although abundant, seemed to be in a mature stage, as no new ones were present; neither was there any proof of division, although some had the appearance of having divided (fig. 10). It was quite evident that it would be necessary to collect material at different times of the year. This was done, the collections covering a period from July 1, 1921, to February 1, 1922.

The first new formations (figs. 2, 3) were found in material collected the second week of December. After this time the formations increased in number, some vessels being completely filled by February 1 (fig. 6). Sections showed tyloses entering the tracheae from adjoining parenchyma cells (figs. 2, 3, 9, 10). It is quite evident that a tylose is formed where the plain pit of a parenchyma cell comes in contact with a bordered pit of a trachea. This, however, would localize tyloses, as parenchyma pits formed in the spring are radial, while those formed in the fall are tangential (7).

Tyloses increased in size, their walls became very thick (fig. 4), and they were separated from the parent cells by cross walls. After a considerable increase in size, mitotic figures began to appear (fig. 11). These were found in different stages, including the telophase (fig. 12) where the wall is formed. More work should be done as to localization and mitotic figures.

Tyloses have many pits in their walls, material taken in January having tyloses in which they were abundant (figs. 7, 8). It is quite evident that intertylosing is a natural consequence where two tylose pits meet (figs. 5, 7). Many tyloses originate from other tyloses by means of these pits (figs. 7, 8).

Just outside the pith are several rows of cells which seem to present a peculiar lateral tylosing, but this has not been established. These cells have much the appearance of the anomalous secondary wood of *Lyginodendron*, and in places look much like phellogen. Their study is difficult because of a brown coloration which is due to an oxidase, not a pigment. This coloration is not continuous, being scattered promiscuously throughout the stem, but always confined to these cells and adjacent tissues.

Although *Menispermum* usually twines around a small tree or shrub, it may extend from one to another, and as these sway in the wind, such severe strains are given the dependent plant that it is

common to find the medullary rays so ruptured that the vascular bundles become more or less separated, giving the stem the appearance of a cable of separate strands. Nature seems to compensate for this injury by strengthening the stem with tyloses.

Summary

1. This paper reports the results of a study of tyloses in *Menispermum*, with reference to time of formation, structure, and behavior.

2. Sections showed no new formations until the second week of December, after which formations were rapid, some vessels being completely filled by February 1.

3. At this time the tyloses were filled with active protoplasm, and in most instances each tylose contained the nucleus which had passed into it, probably facilitating its growth.

4. Intertylosing was common where pits of different tyloses came in contact.

5. That tyloses divide was established by different stages of mitosis.

This study was suggested by Professor CHARLES J. CHAMBERLAIN, to whom sincere appreciation is due for criticism and suggestions.

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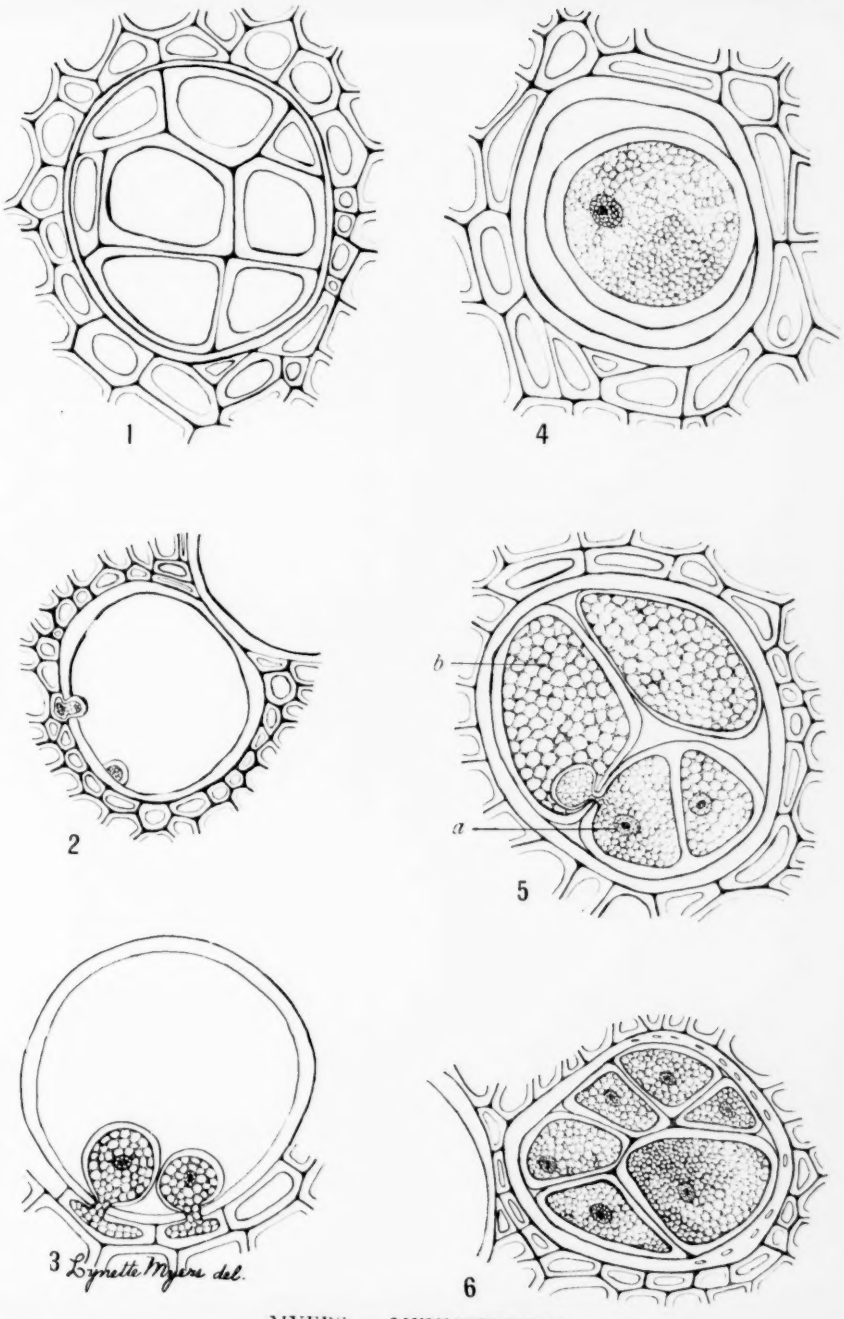
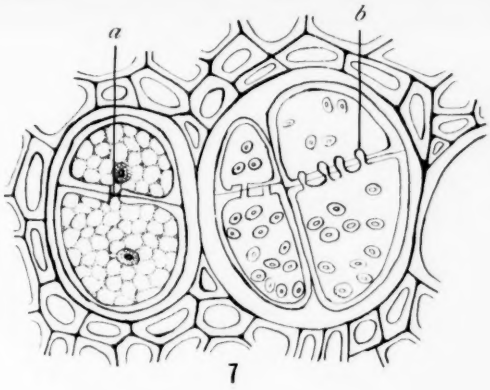


MYERS on MENISPERMUM



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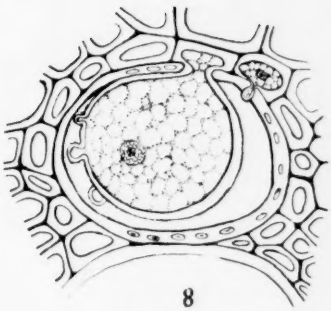

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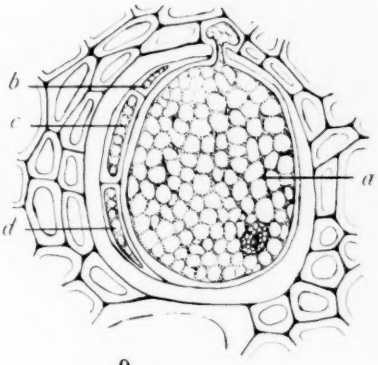

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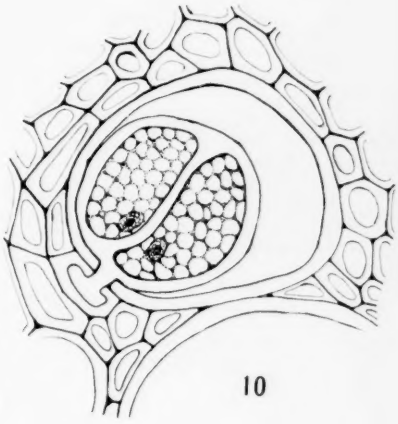

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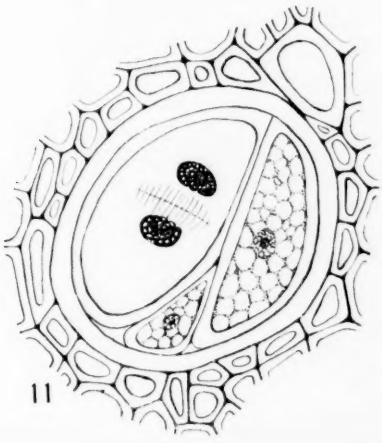

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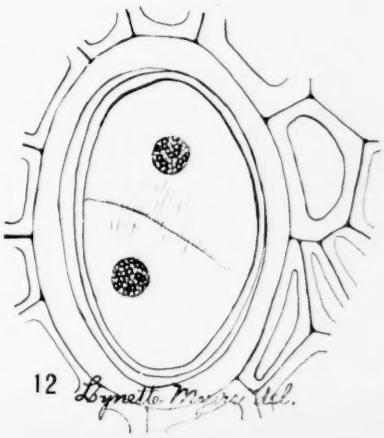

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EXPLANATION OF PLATES XI, XII

FIG. 1.—Tylose situation typical of July or August, vessel crowded with tyloses showing no cell contents.

FIG. 2.—Young tylose with nucleus still in parenchyma cell.

FIG. 3.—Young tyloses containing nuclei.

FIG. 4.—Tylose as it appears first week of January; size greatly increased.

FIG. 5.—Tylose (*a*) forming tylose in an adjoining tylose (*b*).

FIG. 6.—Typical February 1 situation; tracheae filled with tyloses having active cell contents.

FIG. 7.—Tyloses having conspicuous pits; pit of adjoining tyloses meeting (*a*); intertylose formation (*b*).

FIG. 8.—Large tylose from which other tyloses are being formed.

FIG. 9.—Tylose (*a*) from which tyloses (*b*, *c*, *d*) may have originated.

FIG. 10.—Tylose having appearance of having divided.

FIG. 11.—Tylose dividing; mitotic figures near center.

FIG. 12.—Tylose division almost completed; wall being formed.

BRIEFER ARTICLES

DETACHMENT (ELIMINATION) OF CHROMOSOMES IN CYPRIPEDIUM ACAULE

(WITH TWO FIGURES)

In the fall of 1922, three plants of *C. acaule* were removed from the woods to pots in a cold frame. Early in 1923 they were transferred to the greenhouse. On April 3, half of an anther was examined from one plant. Almost all of the young pollen grains had only one nucleus. Some were in the metaphase, however, and among these six groups of ten *V*-shaped chromosomes were counted (fig. 1). One microcyte was found, whose nucleus was about the size of a chromosome. This showed that detachment had occurred in at least one case; therefore more samples were examined. On the next day, most of the pollen grains had finished the division. The whole slide showed only nine cells with ten chromosomes, which, although they differed somewhat in length, were long and *V*-shaped in eight of the cells, and short and thick in one exceptional case. There were also eight cells with nine short and nearly straight chromosomes each (fig. 2). Four microcytes were seen, as well as five micronuclei in otherwise normal grains, but no special search was made for these. Here we have evidence of at least four cases of detachment in the previous maturation divisions, probably at the first division. Another anther-half from the same plant showed most of the microspores with two nuclei. Twelve cells had ten long *V*-chromosomes, and one cell with nine short chromosomes was seen;

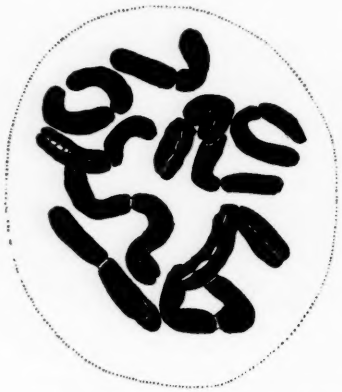

FIG. 1.—Metaphase of first division in pollen grain: ten *V*-shaped chromosomes show constriction at apex of *V;* fissure for longitudinal division obvious at this stage.

while five microcytes and one micronucleus were found without special search.

From the second plant (the third plant did not flower) a sample showed cells with ten long chromosomes, while no microspores with short

FIG. 2.—Three adjacent microspores: left-hand upper cell has completed nuclear division; in right-hand grain division has not begun, there is a micronucleus in cytoplasm, which arose probably from detached chromosome at preceding reduction division; lower pollen grain contains only nine chromosomes, which are comparatively short and straight.

(Both figures drawn with the camera, with a 2.5 mm. apochromatic water-immersion objective, and Wratten light-filter no. 56; preparations in iron-acetocarmine).

chromosomes were seen. (One microspore of double volume, and with a nucleus of double size, pointed to a case of non-reduction.) This flower bud, however, was examined too early to ascertain the presence of 9-chromosome microspores.

An obvious hypothesis is that the sudden change to warmth and the hastening of the flowering had produced these anomalies. It has not yet been shown, however, that they do not exist in the plants in the woods. The shortened state of the chromosomes, and the relative abundance of the 9-chromosome condition in the last cells to be found in the metaphase stage, may lead to the assumption that the microspores with one chromosome lacking were either longer in finishing their division than those with the complete group of ten, or perhaps did not complete their division at all (and so did not produce vegetative and generative nuclei), but aborted, as is the case with microspores with deficient chromosome numbers in the haploid *Datura*, and some other plants.—JOHN BELLING, *Carnegie Institution of Washington, Department of Genetics, Cold Spring Harbor, N.Y.*

GRINDING WOOD SAMPLES FOR ANALYSIS

(WITH ONE FIGURE)

The preparation of samples of woody tissues for the analysis of physiologically important changes in constituents in the past has been a rather difficult task, or has been accomplished only by expensive grinders which all too frequently require large samples for operation. It has been difficult to cut or grind with the ball mill, or other grinding equipment found on the market, such tissues as barberry roots or apple limbs into particles sufficiently small for easy extraction.

The writers adopted the idea of using a pencil sharpener for preparing these hard woody tissues for extraction, the Dexter pencil sharpener manufactured by the Automatic Pencil Sharpener Company of Chicago being used. This sharpener is provided with a strong frame and has good bearings which can be lubricated. There is provided also a convenient container for catching the ground sample. The machine can be operated by hand and costs little. Samples of twigs or split wood up to half an inch in diameter may be ground directly. There is no difficulty in recovering the sample quantitatively after grinding, and the machine can be cleaned quickly and easily.

The hardest dry oak or the softest green twigs may be ground, and each will come out in a finely comminuted condition suitable for extraction by alcohol or other solvent. For the analysis of apple twigs we have used the machine driven by a $\frac{1}{8}$ H.P. motor through a 1–10 reducing pulley. A sample of 100 gm. apple twigs can be ground in less than five

minutes. There is no heating of the cutters, even when grinding hard oak wood at the full capacity of the motor. It is best to press the material into the machine an inch at a time and then allow the motor to pick up speed to clean the cutters. It is possible to grind the material

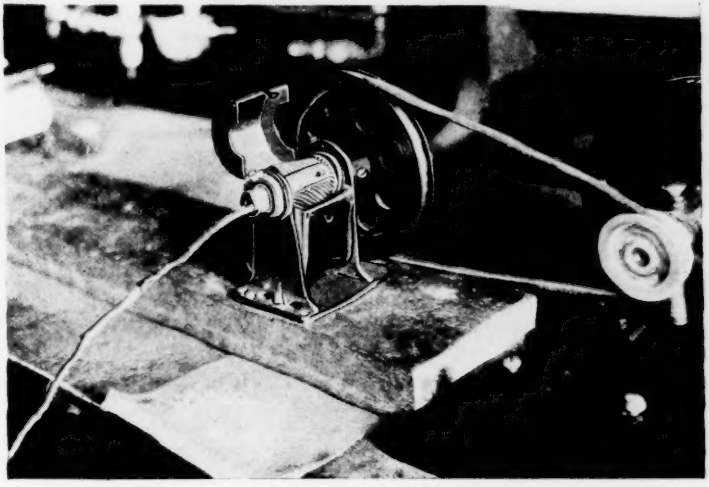

FIG. 1

faster in this manner than by feeding the sticks into the cutters by continuous pressure.—A. C. HILDRETH and R. B. HARVEY, *University Farm, St. Paul, Minn.*

ADVENTITIOUS SHOOTS ON HYPOCOTYL OF FLAX AND TOMATO

(WITH ONE FIGURE)

As a general rule, when the stem of a seedling dicotyledon is cut just above the level of the cotyledons, two branches are formed, one in the axil of each cotyledon. If the stem is cut below the cotyledons, however, the seedling in most plants dies. The result is different, however, in the case of flax.

During some experiments in May, 1920, with flax growing in pots in a greenhouse, as the seedlings were too numerous and as I did not wish to

disturb the roots of the others by pulling up those that were not wanted, I thought it would serve the purpose equally well to behead some of the seedlings with a pair of scissors. Instead of dying they remained perfectly green, however, and at length proceeded to form adventitious buds. The same result has been observed for three years in succession. From one to seven buds may arise on the hypocotyl, but usually only one (or sometimes two) grows out to form a leafy shoot, which may reach a

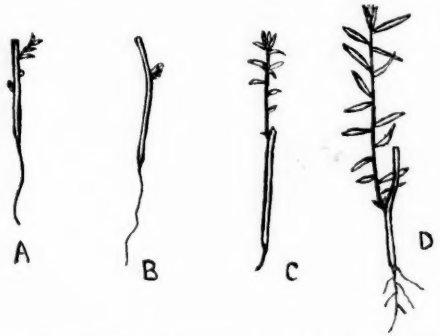

FIG. 1.—Adventitious buds on hypocotyl of flax: in *B* one bud is developing; in *A* two buds and one leafy shoot are seen; in *C* and *D* later stages of development are seen; *A*, *B*, *C*, natural size; *D*, half natural size.

length of 275 mm. These buds never originate from the callus which covers the cut end of the hypocotyl, but always at a considerable distance below it (fig. 1). The appearance of these adventitious buds is favored by the moist air of the greenhouse, their occurrence being much rarer when the seedlings are growing in the open.

The formation of similar buds has also been observed on tomato seedlings in the same greenhouse, but they are much rarer in their occurrence in this plant, and in all the seedlings examined they originated from the callus covering the cut surface of the hypocotyl.—J. ADAMS, *Central Experimental Farm, Ottawa, Canada.*

CURRENT LITERATURE

BOOK REVIEWS

Some notable European botanical textbooks

During the past two years there has been such a thorough revision of some standard European botanical texts, that they deserve at least the attention accorded to new editions. Among these, a prominent place should be given to WETTSTEIN'S *Handbuch der Systematischen Botanik*;[1] *Lehrbuch der Botanik*, by FITTING, JOST, SCHENCK, and KARSTEN;[2] *Das Botanische Praktikum*, by STRASBURGER;[3] and to MASSART'S *Éléments de Biologie Générale et de Botanique*.[4]

WETTSTEIN'S book appeared in 1901, and the second edition, much enlarged and improved, came in 1911. It had been out of print for some time when the first volume of the third edition was published in 1923, followed by the second volume in 1924. The title would lead one to expect a treatise on taxonomy, but the whole field of modern morphology is presented, with emphasis on critical details of life histories, thus laying a foundation for a phylogenetic classification. The descriptions are accompanied by a wealth of illustrations seldom seen in a textbook. While many will not approve the position assigned to one group or another, all must admit that the assignments have been made after an exhaustive study of the literature, in addition to an extensive first hand knowledge of plants.

WETTSTEIN divides the whole plant kingdom into seven great lines, or phyla, between which phylogenetic relationship has not been proved. The content of some of these is so evident that no comment need be added. The phyla are as follows: I. Myxophyta; II. Schizophyta; III. Zygophyta; IV. Phaeophyta; V. Rhodophyta (including Bangiae and Florideae); VI. Euthallophyta (including Chlorophyceae, Phycomycetes, Ascomycetes, Basidiomycetes, Fungi Imperfecti, and Lichens); VII. Cormophyta (including Bryophytes, Pteridophytes, Gymnosperms, and Angiosperms). The series of groups used throughout is phylum (Stamm), division (Abteilung), class, order,

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[1] WETTSTEIN, R., Handbuch der Systematischen Botanik. 3d ed. pp. viii+1017. Pt. I, 1923; Pt. II, 1924. Leipzig & Vienna: Franz Deuticke.

[2] FITTING, H., JOST, L., SCHENCK, H., and KARSTEN, G., Lehrbuch der Botanik für Hochschulen. 16th ed. 8vo. pp. vi+685. Jena: Gustav Fischer. 1923.

[3] STRASBURGER, E., and KOERNICKE, M., Das Botanische Praktikum. 7th ed. 8vo. pp. viii+883. Jena: Gustav Fischer. 1923.

[4] MASSART, J., Éléments de Biologie Générale et de Botanique. 8vo. pp. ix+406 (2nd vol). Brussels: Lamertin. 1923.

family, genus, species. There will probably be more difference of opinion in regard to the subdivisions of the phylum VII than to any of the others. Under the Bryophyta there are two classes, the mosses and liverworts, with the mosses placed first, a sequence which will hardly meet the approval of morphologists. Under the Pteridophyta, Lycopodineae and Isoetineae are classes of equal rank. Some of us would regard Lycopodiales as an order with Isoetaceae as a family under that order. Sphenophyllales is treated as an order under the class Equisetineae. While I believe the two assemblages are closely related, I should prefer to treat them as coordinate orders. The "Cycadofilicinae" are placed under the Pteridophytes. This position is questioned, for the seeds are highly developed and the author himself recognizes gymnosperm characters. So long as gymnosperms are defined as they now are, even in WETTSTEIN'S diagnosis, the Cycadofilicales should be regarded as an order of the gymnosperms.

The clear and interesting text, the extensive bibliographies, the splendid illustrations, and the careful work of a veteran botanist have produced a book which will be useful to morphologists, physiologists, and ecologists, and should suggest modern ideas of phylogeny to the professional taxonomist.

Das Botanische Praktikum made its appearance nearly forty years ago, and four editions were published during STRASBURGER'S lifetime. In the fourth edition, much of the revising was done by KOERNICKE, who is responsible for all changes and improvements since that time. The present edition, the seventh, has been thoroughly revised and some portions have been entirely rewritten. Originally this book was intended to enable the student to teach himself the methods of preparing plant material for microscopic study. This object has been maintained, and microtechnique, as it has been developed at the Bonn school, is well presented, for KOERNICKE not only had the advantage of a long and intimate association with STRASBURGER, and with advanced students of many nationalities who came to study with the great botanist, but he has developed his own methods and has become a very independent technician.

In addition to directions for making slides and using all kinds of apparatus connected with microscopy, there are directions for obtaining material and studying it, so that the work is really a textbook of morphology and microchemistry, as well as a textbook of technique. Since there is still some difficulty in obtaining botanical literature, the author asks that papers involving technique and other features covered by the book be sent to him for use in future editions.

The "Bonn text," the first edition of which, by STRASBURGER, NOLL, SCHENCK, and SCHIMPER, appeared in 1894, has now reached its sixteenth edition. SCHENCK is the only one of the original authors now living. SCHIMPER was replaced by KARSTEN, who has written the special morphology of the seed plants, ever since the sixth edition appeared in 1903. NOLL wrote the physiology for the first nine editions, and at his death was succeeded by JOST. STRASBURGER, who planned the textbook and wrote the morphology of the first eleven

editions, died in 1912, since which time that part has been written by FITTING. Throughout the thirty years the general plan has been maintained, and through the numerous editions the authors have endeavored to keep the book up-to-date, rather than to introduce any radical changes.

It would seem better to combine whatever morphology there is in the first part with the morphology given in the third and fourth parts, and to amplify the ecological anatomy and physiology of the first part so as to make a section on ecology. As it stands, ecology has no adequate presentation, and alternation of generations and phylogeny, while receiving some mention, do not make such an impression as they would if presented in connection with the various groups. The physiological section, to one who is only a morphologist, is interesting and valuable, for it presents a modicum of physiology which every morphologist ought to know. On the whole, the book, with its 849 illustrations, is indispensable, and will continue to hold its high place.

MASSART's book is a general text, of college grade, covering morphology, physiology, ecology, paleobotany, and geographic botany. The reviewer has seen only the second volume, which begins with the Bryophytes, in which the *Anthoceros* assemblage is treated as a group coordinate with the Hepaticae and Musci. The mode of treatment of the various groups may be illustrated by the Pteridophytes and Phanerogams, the vegetative structures of which are treated together. There are two general headings, vegetative structures and reproductive structures. Under the first there are four topics, histology, the root, the stem, and the leaf. The root then, for example, is treated under the headings anatomy and external morphology, and anatomy and internal morphology. The internal morphology is then considered under primary structure, origin of primary structure, origin of the vegetative point, and secondary structures. Other features are treated similarly, and remarks on physiology and ecology are everywhere interspersed, although 157 pages are devoted to these fields in part IV. Under the heading "Origin of primary structure," while there is nothing new, the presentation is good and it is interesting to note that this subject is receiving attention.

Like WETTSTEIN, MASSART treats the Cycadofilicales under the Pteridophytes, as a group coordinate with the Filicales, Equisetales, and Lycopodiales. Unless the seed be abandoned as a basis of classification, the reviewer does not see how this group can be placed anywhere except under the gymnosperms. While I have no doubt that the Cycadofilicales developed from heterosporous Filicales, and that development was gradual, with the first seeds very small and doubtless hard to distinguish from large megaspores; nevertheless, when the seed condition was once attained the gymnosperm level was reached, and the fernlike seed plant should be ranked as a gymnosperm.

The book will be useful for reference, and it gives some idea of the general trend of botany in Belgium. The second volume is well illustrated, with 563 figures, many of which are new. Those which are copied are credited, but there is no bibliography.—C. J. CHAMBERLAIN.

A flora of the White Mountain region

Botanists have a professor of Greek to thank for one of the most substantial and scholarly local floras that has appeared for a long time. Dr. A. S. PEASE, formerly of the University of Illinois, now of Amherst College, became deeply interested in the plants of the White Mountains during his student days at Harvard. In 1907 he formulated a plan for the systematic study of the region, to culminate in the present publication.[5] Before his investigations, interest in the botany of Coös County (an area of 1798 square miles, comprising the northern end of New Hampshire) had almost been confined to Mount Washington and the other mountains of the Presidential Range. With some assistance from other members of the New England Botanical Club, Professor PEASE has pursued a program of field work in Coös County every summer, except one, since 1906, gradually completing his records for the less frequented and previously unbotanized parts. By extending his work beyond the higher mountainous area, the author has been better able to trace the geographic relationships of the several components of the flora. For instance, he has traced various southern species up the valleys of the Connecticut, Androscoggin, and Saco rivers, the chief migration routes for southern types, and has located the barriers to their northward extension.

The title of the *Coös Flora* emphasizes that the author has not confined his attention strictly to the White Mountains. One wonders, however, how many botanists and geographers outside of New England identify the White Mountains with Coös County at all. The definiteness of the title may conceal the work from many who would surely find it valuable. Coös County includes all of the markedly alpine part of the White Mountains, and its flora is therefore of interest to everyone who is interested in phytogeography.

Before the publication of this *Flora* it could be said that no other part of the country as much visited by botanists was as imperfectly known. The fact that the height of interest in the White Mountain flora came before the development of modern critical and intensive methods resulted in a dearth of scholarly publication later on. There was doubtless a feeling that everything that could be said had been said somewhere, sometime, but as a matter of fact the literature was both inaccessible and inadequate.

The older publications were in some cases so inaccurate and careless that they were merely pitfalls for unwary compilers. No published records were accepted for the new flora without conscientious verification, either through searching out old herbarium specimens for critical identification or through rediscovery of the species in the field. The utmost that could be gleaned from the early literature was utilized, but only if the data could be verified. As a result, PEASE's *Flora* ranks with the best that have been produced. In some groups, as, for example, in *Crataegus*, additional species will doubtless be discovered, especially in the northern part of the county. Only in this one genus

[5] PEASE, A. S., Vascular flora of Coös County, New Hampshire. Proc. Boston Soc. Nat. Hist. **37**:39–388. *pls. 5–11.* 1924.

have the determinations (made at the Arnold Arboretum) been accepted by the author without critical study of his own. In other critical genera, as in *Rubus*, the author acknowledges aid from Professor FERNALD, and the whole work shows the influence of the Gray Herbarium. The authenticating specimens are mostly in the Herbarium of the New England Botanical Club (containing PEASE's own collections), the Gray Herbarium, and several other New England collections. In addition, the National Herbarium and the Herbarium of the New York Botanical Garden have been utilized.

An excellent phytogeographical and ecological discussion precedes the systematic list. Topography, soil, and climate receive sufficient consideration. The presentation of a twofold classification of habitats, separating those essentially natural from those modified by man, gives occasion for comments upon the previous ecological work in the region. The habitat lists are unusually full, and of course have an accuracy by no means usual.

There is a new summary of the broad relations of the alpine flora, in which it is shown that the endemic element is negligible, consisting only of four plants, a willow, *Salix Peasei*, which is probably a hybrid of *S. herbacea* and *S. Uva-Ursi*, and therefore may be expected in other arctic-alpine regions where the widely distributed parents occur, *Houstonia coerulea* var. *Faxonorum*, a variety of a wide spread lowland plant, and two well marked species, *Geum Peckii* and *Potentilla Robbinsiana*. If, following the history of the other supposed endemics, the latter species were yet to be discovered elsewhere, no ancient endemics whatever would remain of the long list that used to be quoted.

A bibliography, a list of botanical collectors chronologically arranged, and tables of distributional statistics close the introductory portion. The catalogue which follows gives detailed records of localities and exsiccatae for 1297 distinguishable species, varieties, and forms. There are a few well chosen plates illustrating some of the more interesting species. If the flora could have been expanded, additional illustrations of the same character and a series of plates illustrating typical habitats might well have been added. Perhaps there will be a second edition, or a series of more detailed specialized papers to come. As it stands, the *Flora of Coös County* is a model of painstaking local botany. All systematists will be grateful to the Boston Society of Natural History for undertaking its publication.—H. H. BARTLETT.

The plastids

A monograph giving a detailed account of the morphology, cytology, composition, physiological activities, and pathology of the plastids has been prepared by SCHÜRHOFF.[6] The volume forms a part of the *Handbook of plant anatomy* now appearing in parts under the editorship of LINSBAUER. This summary of plastid organization and behavior will be useful to students of anatomy and physiology, and the long list of citations in itself will be a great

[6] SCHÜRHOFF, PAUL N., Die Plastiden. 8vo. pp. iv+225. *figs.* 57. Berlin: Borntraeger. 1924.

help to those who wish the original contributions. The work is mainly a compilation, as the author has not published any contributions to our knowledge of the plastids from his own investigations. In bringing together the literature, the European contributions have been well selected; but, as so frequently happens now, not enough attention has been given to the work done outside of European laboratories.

With reference to the last section of the volume, which deals with the pathology of the plastids, including abnormalities in form, chloroses, metaplastic greening, and mosaics, the discussion does not go far enough to be of any considerable help to those studying mosaics, but it gives a general idea of what has been done in Europe. It fails to reflect the present widespread investigation of mosaic diseases in America.

Although the volume has some quite obvious shortcomings, it brings together a great mass of scattered knowledge; and cytologists, anatomists, physiologists, and pathologists will all have occasion to study it, and it will form a valuable part of the completed handbook.—C. A. SHULL.

Handbook of British mosses

A third edition of DIXON'S[7] *Handbook* has just been issued. It has given opportunity to include the additions to the British flora since 1904, the date of the second edition. The first edition was issued in 1896. The plates have been entirely redrawn and reproduced by photography, every figure in the book having been drawn from nature. The introduction contains an account of the structure of mosses, their classification, nomenclature, etc.

The book contains 712 species in 115 genera. The largest genus is *Hypnum* (56 species), followed by *Bryum* (45 species), *Grimmia* (34 species), and *Dicranum* (30 species). *Sphagnum* is represented by 18 species. This new and revised edition was certainly called for, and will prove to be a great help to students of mosses.—J. M. C.

MINOR NOTICES

Permeability

The papers on permeability by STILES, which ran through several volumes of the *New Phytologist*, and which were recently reviewed by DAVIS,[8] have been reprinted in book form as New Phytologist Reprint no. 13.[9] The list of more than 800 literature citations at the close of the book is in itself a valuable contribution to plant physiology. The English authors and publishers are to be congratulated upon their ability and willingness to bring out such summaries of the progress of research in special fields.—C. A. SHULL.

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[7] DIXON, H. N., The student's handbook of British mosses. 3d. ed. xlviii+582. *pls. 63*. London: Weldon and Wesley. 1924.

[8] BOT. GAZ. **78**:245. 1924.

[9] STILES, WALTER, Permeability. 8vo. pp. viii+296. London: Wheldon & Wesley, Ltd. 1924.

NOTES FOR STUDENTS

The hydrogen ion.—Miss Brooks[10] finds *Bacillus butyricus* and *B. subtilis* very sensitive to the reaction of the growth medium. *B. butyricus* produces carbon dioxide most rapidly at P_H 7, *B. subtilis* at P_H 6.8. As the growth medium is made more acid or alkaline, there is a progressive reduction in the amount of carbon dioxide produced. Spontaneous recovery follows the addition of alkali to the growth medium. Alkali must be added to cause recovery after the addition of acid, and then the recovery is not complete unless little acid was added.

Working under Jones at Wisconsin, Sherwood[11] has studied the relation of the soil reaction to the development of the *Fusarium* wilt of the tomato. The percentage of diseased plants was greatest in the more acid soils. The critical time of infection seemed to be within the first three days following germination. The experiment indicated that the fungus is able to grow within the range of hydrogen ion concentrations likely to occur in the soil. A P_H of 2 seemed to be required to prevent spore germination. These two papers again illustrate the sensitivity of the lower organisms to the reaction of the growth.—S. V. Eaton.

Hepaticae of Switzerland.—Meylan[12] has published a very full and admirable account of the liverworts of Switzerland. The prefatory pages include a history of research in the group, a list of publications dealing with Swiss material, an account of the organography and its terminology, the biology of the group, the variability and value of species, the ecological associations, and the liverwort regions of Switzerland.

The taxonomic presentation furnishes a good illustration of the relative abundance of the liverwort groups. The author recognizes in Switzerland 69 genera and 260 species. Of these the Jungermanniales include 52 genera and 223 species; the Marchantiales 16 genera and 22 species; the Anthocerotales a single genus with 5 species. By far the largest genera are *Lophozia* (28 species), *Scapania* (26 species), and *Cephaloziella* (18 species). It is interesting to note that only a single new genus (*Cladopus*) and species (in *Grimaldia*) are proposed.—J. M. C.

Distribution of marine algae.—A recent contribution by Svedelius[13] is particularly welcome on account of the scanty literature dealing with either

[10] Brooks, Matilda M., The effect of hydrogen ion concentration on the production of carbon dioxide by *Bacillus butyricus* and *B. subtilis*. Jour. Gen. Physiol. **4**:177–186. *figs.* 3. 1921.

[11] Sherwood, E. C., Hydrogen ion concentration as related to the *Fusarium* wilt of the tomato. Amer. Jour. Bot. **10**:537–552. 1923.

[12] Meylan, Ch., Les Hepatiques de la Suisse. Beitrage Kupt. der Schweiz. **6**:1–318. *figs.* 213. 1924.

[13] Svedelius, Nils, On the discontinuous geographical distribution of some tropical and subtropical marine algae. Arktiv. för Botanik **19**1: pp. 70. 1924.

the ecological or floristic distribution of marine algae. In his introduction, he comments on the unsatisfactory character of our present knowledge, and on the futility of attempting to solve the problems of the discontinuous distribution of various forms on the basis of existing conditions. He cites his explanation, given in 1905, of the similarity of the West Indian and Indo-Pacific floras based on the evidence that the Pacific Ocean and the Caribbean Sea were formerly continuous. He then gives details, illustrated by maps, of the distribution of various families and genera which support the hypothesis. Similar evidence supports the hypothesis that the Mediterranean has received several forms from the south during Tertiary times, when an open connection with the Indian Ocean existed.—GEO. D. FULLER.

Vegetation of Canada.—There has recently appeared a decidedly good map of the principal plant formations of Canada.[14] The ecological viewpoint is taken and the mapping seems to be very accurate. The forest types recognized are the northern hygrophytic evergreen, the northern conifer, the northern mixed, the eastern conifer, the hardwood, the mixed, and the Carolinian. In addition, the transitional grove belt, the prairie grassland, and the cleared portion of the mixed forest belt are delimited. It would be an improvement if an attempt had been made to confine the map entirely to the native vegetation, although the area cleared and farmed is principally from the mixed hardwood-conifer and Carolinian formations. This latter area is limited to the portion of Ontario south of the Georgian Bay possessing originally a forest cover similar to that of Ohio and western New York.—GEO. D. FULLER.

Translocation.—By means of girdling and defoliation experiments, CURTIS[15] has offered excellent evidence that there is inadequate longitudinal translocation of foods by the xylem of woody plants, and that the longitudinal movement of the foods is mainly in the phloem. "Although large amounts of carbohydrates are stored in xylem tissues, there is no appreciable longitudinal transfer of sugars through these tissues."—WM. CROCKER.

Cyanogenesis of Sudan grass.—MENAUL and DOWELL[16] find that Sudan grass contains about one-third as much hydrocyanic acid as the grain sorghums. The quantity is greatest in the young plant, and decreases rapidly as the plant matures.—WM. CROCKER.

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[14] LYNCH, F. C. C., Map of the southern portion of the Dominion of Canada indicating vegetation and forest cover. 20×35 inches. Dept. of Interior. Ottawa, Canada. 1924.

[15] CURTIS, O. F., The upward translocation of foods in woody plants. I. Tissues concerned in translocation. Amer. Jour. Bot. 7:101–124. 1920.

[16] MENAUL, P. and DOWELL, C. T., Cyanogenesis of Sudan grass: a modification of the Francis-Connell method of determining hydrocyanic acid. Jour. Agric. Res. 18:447–450. 1920.

GENERAL INDEX

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